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Applicants hereby submit copies of the following priority documents, certified by the EPO, in connection with the above-referenced patent application:

1. GB 9520486.3 (6 October 1995); and
2. GB 9601081.4 (19 January 1995).

Accordingly, it is believed that no fees are required by entry of this paper. However, the Commissioner is hereby authorized to charge any necessary fees, or to credit any overpayment, to Deposit Account 08-3038.

Respectfully submitted,

HOWREY LLP

Dated: November 08, 2007

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Les documents ci-annexés sont conformes aux documents figurant dans le dossier de la demande de brevet dont le numéro est indiqué ci-dessous (règle 94(4) CBE).

Patentanmeldung Nr. Patent application No. Demande de brevet n°

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)*

5781992002

If the applicant is a corporate body, give the country/state of its incorporation

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4. Title of the invention

SPECIFIC BINDING MEMBERS FOR HUMAN TRANSFORMING GROWTH FACTOR BETA; MATERIALS AND METHODS

5. Name of your agent *(if you have one)*

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Patents ADP number *(if you know it)*

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
GREAT BRITAIN	9520486.3	6 OCTOBER 1995

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SPECIFIC BINDING MEMBERS FOR HUMAN TRANSFORMING GROWTH FACTOR BETA; MATERIALS AND METHODS

This invention relates to specific binding members for human transforming growth factor (TGF) beta and materials and methods relating thereto. In particular, it relates to specific binding members comprising antibody binding domains; for example, human antibodies. Human antibodies against human TGFβ may be isolated and utilised in the treatment of disease, particularly fibrotic disease and also immune/inflammatory diseases. The isolation of antiself antibodies from antibody segment repertoires displayed on phage has been described (A.D.Griffiths et al. EMBO J. 12, 725-734, 1993; A. Nissim et al. EMBO J. 13, 692-698, 1994; A.D. Griffiths et al. 13, 3245-3260, 1994; C.Barbas et al. Proc. Natl. Acad. Sci. USA 90, 10003-10007 1993; WO93/11236).

TGFβ is a cytokine known to be involved in many cellular processes such as cell proliferation and differentiation, embryonic development, extracellular matrix formation, bone development, wound healing, hematopoiesis and immune and inflammatory responses(A.B. Roberts & M. Sporn 1990 pp419-472 in Handbook of Experimental Pharmacology eds M.B. Sporn & A.B. Roberts, Springer Heidelberg; J.Massague et al.Annual Rev. Cell Biol. 6, 597-646, 1990).

The accumulation of excessive extra-cellular matrix is associated with various fibrotic diseases.

Thus there is a need to control agents such as TGFβ1 and TGFβ2 to prevent their deleterious effects in such diseases and this is one application of human antibodies to human TGFβ.

The modulation of immune and inflammatory responses by TGFbetas includes (i) inhibition of proliferation of all T-cell subsets (ii) inhibitory effects on proliferation and function of B lymphocytes (iii) down-regulation of natural-killer cell activity and the T-cell response (iv) regulation of cytokine production by immune cells (v) regulation of macrophage function and (vi) leucocyte recruitment and activation.

A further application of antibodies to TGFβ may be in the treatment of immune/inflammatory diseases such as rheumatoid arthritis, where these functions need to be controlled.

It is a demanding task to isolate an antibody fragment specific for TGFβ of the same species. Animals do not normally produce antibodies to self antigens, a phenomenon called tolerance (G.J. Nossal Science 245, 147-153, 1989). In general, vaccination with a self antigen does not result in production of circulating antibodies. It is therefore difficult to raise human antibodies to human self antigens. There are also in addition, ethical problems in vaccinating humans. In relation to the raising of non-human antibodies specific for TGFβ, there are a number of

problems. TGFβ is an immunosuppressive molecule and further, there is strong conservation of sequence between human and mouse TGFβ molecules. Mouse and human TGFβ1 only differ by one amino acid residue, an alanine (human) to serine (mouse) change at a buried residue (R.Derynck et al. J.Biol. Chem. 261, 4377-4379, 1986). This makes it difficult to raise antibodies in mice against human TGFβ. Further, any antibodies raised may only be directed against a restricted set of epitopes.

Polyclonal antibodies binding to human TGFβ1 and human TGFβ2 against both neutralising and non-neutralising epitopes have been raised in rabbit (Danielpour et al. Growth Factors 2 61-71, 1989; A. Roberts et al. Growth Factors 3, 277-286, 1990), chicken (R&D Systems, Minneapolis) and turkey (Danielpour et al. J. Cell Physiol. 138, 79-86, 1989). Peptides representing partial TGFβ sequences have also been used as immunogens to raise neutralising polyclonal antisera in rabbits (W.A Border et al. Nature 346, 371-374, 1990; K.C. Flanders Biochemistry 27, 739-746, 1988). In addition there have been limited reports of isolation of mouse monoclonals against TGFβ. Following immunisation with bovine TGFβ2 (identical to human TGFβ2), three non-neutralising monoclonal antibodies were isolated that are specific for TGFβ2 and one neutralising antibody that is specific for TGFβ1 and TGFβ2 (J.R. Dasch et

al. J. Immunol. 142, 1536-1541, 1989). In another report, following immunisation with human TGFβ1, neutralising antibodies were isolated which were either specific for TGFβ1 or cross-reacted with TGFβ1, TGFβ2 and TGFβ3 (C. Lucas et al. J.Immunol. 145, 1415-1422, 1990).

This application discloses the first isolation of human antibodies directed against human TGFβ1 and against humanTGFβ2.

Phage antibody technology (WO92/01047; PCT/GB92/00883; PCT/GB92/01755; WO93/11236) offers the ability to isolate directly human antibodies against human TGFβ. In application WO93/11236 the isolation of antiself antibodies from phage display libraries was disclosed and it was suggested that antibodies specific for TGFβ could be isolated from phage display libraries.

The present application shows that antibodies of differing specificities for TGFβ molecules may be isolated. TGFβ1, TGFβ2 and TGFβ3 are a closely related group of cytokines. They are dimers consisting of two 112 amino acid monomers joined by an interchain disulphide bridge. TGFβ1 differs from TGFβ2 by 27 mainly conservative changes and from TGFβ3 by 22 mainly conservative changes. These differences have been related to the 3D structure (M.Schlunegger & M.G.Grutter Nature 358, 430-434, 1992). The present applicants have isolated antibodies which are

essentially specific for TGFβ1 (very low cross-reactivity with TGFβ2); antibodies which are essentially specific for TGFβ2 (very low cross-reactivity TGFβ1); and antibodies which bind both TGFβ1 and TGFβ2. Hence, these three different types of antibodies, each type with distinctive binding specificities must recognise different epitopes on the TGFβ molecules. These antibodies have low cross-reactivity with TGFβ3 as assessed by binding studies using biosensor assays (e.g.BIACore™), ELISA and radioreceptor assays.

It has further been demonstrated by the applicants that antibodies specific for TGFβ can be isolated from libraries derived from different sources of immunoglobulin genes: from repertoires of natural immunoglobulin variable domains; and synthetic repertoires derived from germline V genes combined with synthetic CDR3s. The properties of these antibodies in single chain Fv and whole IgG4 format are described.

As noted above WO93/11236 suggested that human antibodies directed against human TGFβ could be isolated from phage display libraries. The applicants show that the phage display libraries from which antiself antibodies were isolated in WO93/11236 may be utilised as a source of human antibodies specific for human TGFβ. For instance, in example 1 of the present application, the antibody 1A-E5 specific for TGFβ1 and

the antibodies 2A-H11 and 2A-A9 specific for TGFβ2 were isolated from the 'synthetic library' described in examples 5 to 7 of WO93/11236 and in Nissim et al. (1994; supra). Also, the phage display library derived from peripheral blood lymphocytes (PBLs) of an unimmunised human (examples 1 to 3 of WO93/11236) was the source for the antibody 1B2 specific for TGFβ1. Phage display libraries made subsequently utilising antibody genes derived from human tonsils and bone marrow, have also provided sources of antibodies specific for human TGFβ. Thus human TGFβ is an example of a human self antigen to which antibodies may be isolated from 'large universal libraries'. Human antibodies against human TGFβ with improved properties can be obtained by chain shuffling for instance combining the VH domains of antibodies derived from one library with the VL domains of another library thus expanding the pool of VL partners tested for each VH domain. For instance, the antibodies 6B1, 6AH and 6H1 specific for TGFβ2 utilise the 2A-H11 VH domain isolated from the 'synthetic library' combined with a light chain from the PBL library.

Thus the VH and VL domains of antibodies specific for TGFβ can be contributed from phage display libraries derived from rearranged V genes such as those in PBLs, tonsil and bone marrow and from V domains derived from cloned germline V segments

combined with synthetic CDRs. There are also shown to be a diverse range of antibodies which are specific for TGFβ1 or TGFβ2. The antibodies which have been isolated both against TGFβ1 and TGFβ2 have mainly utilised V genes derived from VH germlines of the VH3 family. A wider variety of light chain variable regions have been used, of both the lambda and kappa types.

Individual antibodies which have been isolated have unexpectedly advantageous properties. For example, the antibodies directed against TGFβ2 (6H1, 6A5 and 6B1) have been shown to bind to TGFβ2 with slow off-rates (off-rate constants k_{off} of the order of 10^{-3} s^{-1}) to neutralise TGFβ2 activity in in vitro assays and to be potent in in vivo applications. The properties of these antibodies may make them particularly suitable for therapeutic applications. The fact that these antibodies share the same heavy chain, shows that VH domains can be effective with a number of different light chains, although there may be differences in potency or subtle changes of epitope with different light chains. The antibodies directed against TGFβ1 (1AE5, 1AH6 and 1B2 and their derivatives) also have unexpectedly advantageous properties. Antibody 27C1/10A6 derived from 1B2 by chain shuffling, spiking and conversion into whole antibody IgG4 , has been shown to be potent in an in vitro scarring model. The VH domain of this antibody

was derived by site directed 'spiking' mutagenesis from the parent antibody 7A3. A large number of spiked clones were obtained which show similar properties in in vitro assays. There can be a number of changes in CDR3 of the VH compared to 27C1, for instance, 28A-H11 differs in 7 of the 14 positions, 2 of which are non-conservative changes. Thus there may be up to 50% of the residues in the VH CDR3 changed without affecting binding properties.

Antibodies specific for human TGFβ1 and human TGFβ2 have been shown to be effective in animal models for the treatment of fibrotic diseases and other diseases such as rheumatoid arthritis where TGFβ is overexpressed. Antibodies against TGFβ have been shown to be effective in the treatment of glomerulonephritis (W.A Border et al. Nature 346, 371-374, 1990); neural scarring (A. Logan et al. Eur. J. Neurosci. 6, 355-363, 1994); dermal scarring (M. Shah et al. Lancet 339, 213-214 1992; M.Shah et al. J.Cell Science 107, 1137-1157, 1994; M. Shah et al. 108, 985-1002, 1995); lung fibrosis (S.N. Giri et al. Thorax 48, 959-966, 1993); arterial injury (Y.G. Wolf, L.M. Rasmussen & E. Ruoslahti J. Clin. Invest. 93, 1172-1178, 1994) and rheumatoid arthritis (Wahl et al J. Exp. Medicine 177, 225-230, 1993). It has been suggested that TGFβ3 acts antagonistically to TGFβ1 and TGFβ2 in dermal scarring (M.Shah et al. 1995 supra.). Therefore, antibodies to TGFβ1 or TGFβ2 with

apparent low cross-reactivity to TGFβ3, as assessed by binding studies using a biosensor assay (e.g BIACore™), ELISA or a radioreceptor assay, as disclosed in this application, that is to say antibodies which bind preferentially to TGFβ1 or TGFβ2 compared with TGFβ3, should be advantageous in this and other conditions such as fibrotic conditions in which it is desirable to counteract the fibrosis promoting effects of TGFβ1 and TGFβ2.

There are likely to be applications further to the above mentioned conditions, as there are several other in vitro models of disease where antibodies against TGFβ have shown promise of therapeutic efficacy including antibodies directed against TGFβ2 for the treatment of eye diseases such as proliferative retinopathy (R.A. Pena et al. Invest. Ophthalmology. Vis. Sci. 35, 2804-2808, 1994), retinal detachment and post glaucoma drainage surgery. Other diseases which have potential for treatment with antibodies against TGFβ include adult respiratory distress syndrome, cirrhosis of the liver, post myocardial infarction, post angioplasty restenosis, keloid scars and scleroderma.

The use of antibodies against TGFβ for the treatment of diseases has been the subject of patent applications for fibrotic disease (WO91/0478); dermal scarring (WO92/17206); macrophage deficiency diseases (PCT/US93/00998); macrophage pathogen infections

(PCT/US93/02017); neural scarring (PCT/US93/03068); vascular disorders (PCT/US93/03795); prevention of cataract (WO95/13827). The human antibodies against human TGFβ disclosed in this application should be valuable in these conditions.

The applicants show herein that the human antibodies both against human TGFβ1 and against human TGFβ2 can be effective in the treatment of fibrosis in animal models of neural scarring and glomerulonephritis in either single chain Fv and whole antibody format. This is the first disclosure of the effectiveness of antibodies directed only against TGFβ2 as sole treatment in these indications, although some effectiveness of antibodies against TGFβ2 only has been observed in a lung fibrosis model (Giri et al. Thorax 48, 959-966, 1993 supra). The effectiveness of the human antibodies against human TGFβ in treatment of fibrotic disease has been determined by measuring a decrease in the accumulation of components of the extracellular matrix, including fibronectin and laminin in animal models.

TERMINOLOGY

Specific binding member

This describes a member of a pair of molecules which have binding specificity for one another. The members of a specific binding pair may be naturally

derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. This application is concerned with antigen-antibody type reactions.

Antibody

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein having a binding domain which is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric

molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment

consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993).

Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. 4, 446-449 (1993)), eg prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. It may be preferable to use scFv dimers or diabodies rather than whole antibodies. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. Other forms of bispecific antibodies include the single chain "Janusins" described in Traunecker et al, Embo Journal, 10, 3655-3659, (1991).

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E.coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected.

Antigen binding domain

This describes the part of an antibody which

comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antibody binding domain may be provided by one or more antibody variable domains. Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

Specific

This refers to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner. The term is also applicable where eg an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope.

Neutralisation

This refers to the situation in which the binding of a molecule to another molecule results in the abrogation of the biological effector functionof the another molecule.

Functionally equivalent variant form

This refers to a molecule (the variant) which although having structural differences to another molecule (the parent) retains some significant homology and also at least some of the biological function of the parent molecule, e.g. the ability to bind a particular antigen or epitope. Variants may be in the form of fragments, derivatives or mutants. A variant, derivative or mutant may be obtained by modification of the parent molecule by the addition, deletion, substitution or insertion of one or more amino acids, or by the linkage of another molecule. These changes may be made at the nucleotide or protein level. For example, the encoded polypeptide may be a Fab fragment which is then linked to an Fc tail from another source. Alternatively, a marker such as an enzyme, flourescein, etc, may be linked.

The present invention provides a specific binding member which comprises a human antibody antigen binding domain specific for TGFβ1 and/or TGFβ2 and which has low cross reactivity with TGFβ3. The cross-reactivity may be as assessed using any or all of the following assays: biosensor (e.g. BIACore™), ELISA and radioreceptor. The present invention provides specific binding member which comprises a human antibody antigen binding domain specific for TGFβ1 and/or TGFβ2 which binds preferentially to these isoforms compared with TGFβ3.

The TGFβ may be human TGFβ.

The specific binding member may be in the form of an antibody fragment such as single chain Fv (scFv). Other types of antibody fragments may also be utilised such as Fab, Fab', $F(ab')_2$, Fabc, Facb or a diabody (G.Winter & C.Milstein Nature 349, 293-299, 1991; WO94/13804). The specific binding member may be in the form of a whole antibody. The whole antibody may be in any of the forms of the antibody isotypes egIgG, IgA, IgE, and IgM and any of the forms of the isotype subclasses eg IgG1 or IgG4.

The specific binding memeber may also be in the form of an engineered antibody eg bispecific antibody molecules (or fragments such as $F(ab')_2$) which have one antigen binding arm (ie specific binding domain) against TGFβ and another arm against a different specificity. Indeed the specific binding members directed against TGFβ1 and/or TGFβ2 described herein may be combined in a bispecific diabody format. For example the antibodies 31G9 directed against TGFβ1 and 6H1 directed against TGFβ2 may be combined to give a single dimeric molecule with both specificities.

The binding domain may comprise part or all of a VH domain encoded by a germ line gene segment or a re-arranged gene segment. The binding domain may comprise part or all of either a VL kappa domain or a VL lambda domain.

The binding domain may comprise a VH3 gene

sequence of one of the following germ lines; the DP49 germ line; the DP53 germ line; the DP50 germ line; the DP46 germ line; or a re-arranged form thereof.

The specific binding member may neutralise the in vitro and/or in vivo effect of TGFβ.

The specific binding member may be a high affinity antibody.

The binding domain may comprise part or all of a VH domain having either an amino acid sequence as shown in Fig 1(a)(i) to (iv) or Fig 1(c)(i) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VH domain encoded by either a nucleotide sequence as shown in Fig 1(a)(i) to (iv) or Fig 1(c)(i) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as shown in Fig 1(a)(v) or Fig 1(b) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VL domain encoded by either a nucleotide sequence as shown in Fig 1(a)(v) or Fig 1(b) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VH domain having a variant form of the Fig 1(a)(i) amino acid, the variant form being one of those as

provided by Fig 3.

The binding domain may comprise part or all of a VH domain having either an amino acid sequence as shown in Fig 2(a)(i) to (iii),(v) and (vi) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VH domain encoded by either a nucleotide sequence as shown in Fig 2(a)(i) to (iii),(v) and (vi) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as shown in Fig 2(a)(iv) or Fig 2(b)(i) to (v) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VL domain encoded by either a nucleotide sequence as shown in Fig 2(a)(iv) or Fig 2(b)(i) to (v) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may be specific for both TGFβ1 and TGFβ2. The binding domain may be specific for both human TGFβ1 and human TGFβ2. The specific binding member may be in the form of scFv.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as shown in Fig 4 or a functionally equivalent variant

form of said amino acid sequence. The binding domain may comprise part or all of a VL domain encoded by either the nucleotide sequence as shown in Fig 4 or a functionally equivalent variant form of said nucleotide sequence.

In particular, the binding domain may comprise one or more CDR (complementarity determining region) with an amino acid sequence shown in any of the figures. In a preferred embodiment, the binding domain comprises one or more of the CDRs, CDR1, CDR2 and/or CDR3 shown in the Figures, especially any of those shown in Figure 19. In a preferred embodiment, the binding domain comprises a VH CDR3 sequence as shown, especially as shown in Figure 19 . Functionally equivalent variant forms of the CDRs are encompassed by the present invention, in particular variants which differ from the CDR sequences shown by addition, deletion, substitution or insertion of one or more amino acids and which retain ability to bind the antigen and optionally one or more of the preferred characteristics for specific binding members of the present invention as disclosed herein. The specific binding member may comprise all or part of the framework regions shown flanking and between the CDRs in the Figures, especially Figure 19, or different framework regions including modified versions of those shown.

So-called "CDR-grafting" in which one or more CDR

sequences of a first antibody is placed within a framework of sequences not of that antibody, e.g. of another antibody is disclosed in EP-B-0239400.

The present invention also provides a polypeptide with a binding domain specific for TGFβ which polypeptide comprises a substantial part or all of either an amino acid sequence as shown in any one of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig 2(b), Fig 4 or a functionally equivalent variant form of a said amino acid sequence. The polypeptide may comprise a substantial part or all of an amino acid sequence which is a functionally equivalent variant form of the Fig 1(a)(i) amino acid sequence, the variant being one of those variants as shown in Fig 3.

A specific binding member according to the invention may be one which competes for binding to TGFβ1 and/or TGFβ2 with any specific binding member which both binds TGFβ1 and/or TGFβ2 and comprises part of all of any of the sequences shown in the Figures. Competition between binding members may be assayed easily *in vitro*, for example by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

In addition to an antibody sequence, the specific binding member may comprise other amino acids, e.g.

forming a peptide or polypeptide, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. For example, the specific binding member may comprise a label, an enzyme or a fragment thereof and so on.

The present invention also provides a polynucleotide which codes for a polypeptide with a binding domain specific for TGFβ which polynucleotide comprises a substantial part or all of a nucleotide sequence which codes for either an amino acid sequence as shown in any one of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig 2(b), Fig 4 or a functionally equivalent variant form of a said amino acid sequence. The polynucleotide may code for a polypeptide with a binding domain specific for TGFβ which polynucleotide comprises a substantial part or all of a nucleotide sequence which codes for an amino acid sequence which is a functionally equivalent variant form of the Fig 1(a)(i) amino acid sequence, the variant being one of those as shown in Fig 3. The polynucleotide may code for a polypeptide with a binding domain specific for TGFβ which polynucleotide comprises a substantial part or all of a either a nucleotide sequence as as shown in any one of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig 2(b), Fig 4 or a functionally equivalent variant form of said nucleotide sequence. The polynucleotide may code for a polypeptide with a binding domain specific for TGFβ which polynucleotide

comprises a substantial part or all a nucleotide sequence which codes for a variant form of the Fig 1(a)(i) amino acid sequence, the variant being one of those as shown in Fig 3.

The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise least one polynucleotide as above.

The present invention also provides a recombinant host cell which comprises one or more constructs as above.

A specific binding member according to the present invention may be made by expression from encoding nucleic acid. Nucleic acid encoding any specific binding member as provided itself forms an aspect of the present invention, as does a method of production of the specific binding member which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid.

The nucleic acid may encode any of the amino acid sequences shown in any of the Figures, or any functionally equivalent form. The nucleotide sequences employed may be any of those shown in any of the Figures, or may be a variant, allele or derivative thereof. Changes may be made at the nucleotide level by addition, substitution, deletion or insertion of

one or more nucleotides, which changes may or may not be reflected at the amino acid level, dependent on the degeneracy of the genetic code.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Plückthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding member, see for recent reviews, for example Reff, M.E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J.J. et al. (1995) Curr. Opinion Biotech 6: 553-560.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as

appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression

of the gene.

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

The present invention also provides a method which comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as above.

Following production of a specific binding member it may be used for example in any of the manners disclosed herein, such as in the formulation of a pharmaceutical or a diagnostic product, such as a kit comprising in addition to the specific binding member one or more reagents for determining binding of the member to cells, as discussed.

The present invention also provides pharmaceuticals which comprise a specific binding member as above, optionally with one or more excipients.

The present invention also provides the use of a specific binding member as above in the preparation of a medicament to treat a condition in which it is advantageous to counteract the fibrosis promoting effects of TGFβ. The condition may be a fibrotic condition characterized by an accumulation in a tissue

of components of the extracellular matrix. The components of the extracellular matrix may be fibronectin or laminin.

The condition may be selected from the group consisting of:

glomerulonephritis
neural scarring
dermal scarring
lung fibrosis
arterial injury
proliferative retinopathy
retinal detachment
adult respiratory distress syndrome
liver cirrhosis
post myocardial infarction
post angioplasty restenosis
keloid scarring
scleroderma
vascular disorders
cataract
glaucoma.

The condition may be neural scarring or glomerulonephritis.

The present invention also provides the use of a specific binding member as above, in the preparation of a medicament to treat an immune/inflammatory disease condition in which it is advantageous to

counteract the effects of TGFβ. Illustrative conditions are rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

The present invention also provides a method which comprises administering to a patient a therapeutically effective amount of a specific binding member as above in order to treat a condition in which it is advantageous to counteract the fibrosis promoting effects of TGFβ. Fibrotic conditions are listed above.

The present invention also provides a method which comprises administering to a patient a prophylactically effective amount of a specific binding member as above in order to prevent a condition in which it is advantageous to prevent the fibrosis promoting effects of TGFβ. Fibrotic conditions are listed above.

The present invention also provides methods which comprise administering to patients prophylactically and/or therapeutically effective amounts of a specific binding member as above in order to prevent or treat an immune/inflammatory disease condition in which it is advantageous to counteract the effects of TGFβ. Illustrative conditions are stated above.

Thus, various aspects of the invention provide methods of treatment comprising administration of a specific binding member as provided, pharmaceutical compositions comprising such a specific binding

member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the specific binding member with a pharmaceutically acceptable excipient.

In accordance with the present invention, compositions provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practioners and other medical doctors. Appropriate doses of antibody are well known in the art; see Ledermann J.A. et al. (1991) Int J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the

present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers,

antioxidants and/or other additives may be included, as required.

Further aspects of the invention and embodiments will be apparent to those skilled in the art. In order that the present invention is fully understood, the following examples are provided by way of exemplification only and not by way of limitation.

Reference is made to the following figures.

Figure 1 shows the DNA and protein sequences of antibodies specific for TGFβ1.

Figure 2 shows the DNA and protein sequences of antibodies specific for TGFβ2.

Figure 3 shows the protein sequences of VH CDR3 of clones derived from 1B2 by 'spiking' mutagenesis.

Figure 4 shows the DNA and protein sequence of the VH and VL domains of VT37.

Figure 5 shows the DNA sequence in the region of the heavy chain VH leader from the vector vhcassette2.

Figure 6 shows a map of the vector pG4D100.

Figure 7 shows the DNA sequence in the region of the light chain VL leader for the vector vlcassette1.

Figure 8 shows a map of the vector pLN10.

Figure 9 shows a map of the vector pKN100.

Figure 10 shows the neutralisation of TGFβ2 activity by whole IgG4 antibodies in an assay using proliferation of the erythroleukaemia cell line, TF1.

Figure 11 shows the neutralisation of TGFβ2 activity by single chain Fv antibodies in an assay using proliferation of the erythroleukaemia cell line, TF1.

Figure 12 shows the effect of treatment of animals with antibodies on neural scarring as measured by the deposition of (a) fibronectin and (b) laminin detected using integrated fluorescence intensity. The graphs show scatter plots of individual animal data points. The bar graph shows the mean of the group.

Figure 13 shoes the results of an ELISA to measure the cross-reactivity of the antibodies 6B1 IgG4 and 6A5 IgG4 with TGFβ isoforms and non-specific antigens.

Figure 14 shows the amount of urinary protein in 24h measured for rat groups A to E in the experimental glomerulonephritis model.

Figure 15 shows the periodic acid Schiff matrix score (derived by measurement of the amount of staining) for rat groups A to E in the experimental glomerulonephritis model.

Figure 16 shows % neutralisation of TGF-β2 anti-proliferative effect on TF1 cells by whole antibodies, 6H1 IgG4, 6B1 IgG2 and the mouse monoclonal from Genzyme, at various concentrations (nM IgG).

Figure 17 shows % neutralisation of TGF-β1 anti-proliferative effect on TF1 cells by whole antibodies,6H1 IgG4, 6B1 IgG2 and the mouse monoclonal

from Genzyme, at various concentrations (nM IgG).

Figure 18 shows % neutralisation of TGF-β3 anti-proliferative effect on TF1 cells by whole antibodies, 6H1 IgG4, 6B1 IgG2 and the mouse monoclonal from Genzyme, at various concentrations (nM IgG).

Figure 19 shows sequences of regions of antibodies directed against TGFβ2 showing CDR sequences in italics: 2A-H11 VH (also known as 6H1 VH); 6B1 VL; 6A5 VL and 6H1 VL.

All documents mentioned herein are incorporated by reference.

List of Examples

Example 1 - Isolation of antibodies specific for TGFβ1, antibodies specific for TGFβ2 and antibodies specific for TGFβ1 and TGFβ2.

Example 2 - Construction of cell lines expressing whole antibodies.

Example 3 - Neutralisation of TGFβ activity by antibodies assessed using in vitro assays.

Example 4 - Inhibition by antibodies of TGFβ binding to receptors.

Example 5 - Prevention of neural scarring using antibodies against TGFβ.

Example 6 - Prevention of glomerulonephritis using antibodies against TGFβ.

Example 7 - Neutralisation by antibodies directed against TGFβ2 of the inhibitory effect of TGFβ isoforms on cells proliferation.

Example 8 - Inhibition by antibodies directed against TGFβ2 of binding of other TGFβ isoforms to receptors measured in a radioreceptor assay.

<u>Example 1 Isolation and characterisation of antibodies binding to TGFβ1 and TGFβ2</u>

<u>1. Identification and Characterisation of Antibodies to Human TGFb-1 by Selection of Naive and Synthetic Phage Antibody Repertoires</u>

Antibody repertoires

The following antibody repertoires were used:

1. <u>Peripheral blood lymphocyte (PBL) library</u> derived from unimmunized human (Marks, J. D., Hoogenboom, H. R. Bonnert, T. P., McCafferty, J., Griffiths, A. D. &

Winter, G. (1991) J. Mol. Biol. 222, 581-597)

2. Synthetic library (Nissim, A., Hoogenboom, H. R., Tomlinson, I. M., Flynn, G., Midgley, C., Lane, D. and Winter, G. (1994) EMBO J. 13, 692-698) derived from cloned human germline VH genes and synthetic CDR3s with a fixed light chain

3. Tonsil library derived from the tonsils of unimmunised humans. Tonsil B cells were isolated from freshly removed (processed within 2 hours) whole tonsils provided by Addenbrookes Hospital, Hills Road, Cambridge, U.K. Each tonsil was processed as follows. Tonsils were placed in a petri dish containing 5ml of PBS and macerated with a scalpel blade to release the cells. The suspension was transferred to a fresh tube and large debris allowed to sediment under gravity for 5 minutes. The cell suspension was then overlaid onto 10mls of Lymphoprep in a 50 ml polypropylene tube (Falcon) and centrifuged at 1000xg 20 minutes at room temperature (no brake) and cells at the interface harvested with a glass pipette. These were diluted to a final volume of 50 ml in RPMI medium at 37^0 C and centrifuged at 500xg for 15 minutes at room temperature. The supernatant was aspirated and the the cells washed another two times with RPMI.

Polyadenylated RNA was prepared from pelleted cells using the "QuickprepTM mRNA Kit" (Pharmacia

Biotech, Milton Keynes, U.K.). The entire output of cells from one tonsil (ca. $1x10^6$ cells) was processed using one Oligo(dT)-Cellulose Spun column and processed exactly as described in the accompanying protocol. MRNA was ethanol precipitated as described and resuspended in 40ml RNase free water.

The cDNA synthesis reaction was set up using the "First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Milton Keynes, U.K.) as follows:

RNA- 20μl (heated to 67 ^{0}C 10 minutes before use)

1st strand buffer-	11μl
DTT solution	1μl
pd(N)$_6$ primer	1μl

After gentle mixing, the reaction was incubated at 37 ^{0}C for 1 hour.

Human VH genes were amplified from tonsil cDNA using the nine family-based back primers (VH 1b/7a -6a back *Sfi* , which introduce a *Sfi* I site at the 5'-end, Table 1) together with an equimolar mixture of the four JH forward primers (JH 1-2, 3, 4-5, 6, for; Marks et al., 1991 supra). Thus, nine primary PCR amplifications were performed. Each reaction mixture (50 μl) comprised 2 μl cDNA template, 25 pmol back primer, 25 pmol forward primers, 250 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCL pH 8.3 and 2.5 u of *Taq* polymerase (Boehringer). The reaction mixture was overlaid with mineral (paraffin) oil and was cycled 30

times (94 ^{0}C for 1 min, 55 ^{0}C for 1 min, 72 ^{0}C for 1 min) using a Techne thermal cycler. The products were purified on a 1% (w/v) agarose gel, isolated from the gel using "Geneclean" (Bio 101 Inc.) and resuspended in 15 μl of water. The amplified VH genes were recombined with human VL genes derived from PBLs (Marks et al., 1991 supra) together with the (Gly_4, $Ser)_3$ linker (Huston, J.S., et al. 1988 Proc Natl Acad Sci U S A. 85: 5879-83) by PCR assembly (Marks et al, 1991 supra). The VH-linker-VL antibody constructs were cloned into the SfiI and NotI sites of the phagemid vector, pCANTAB6 (McCafferty, J., et al. 1994 Appl. Biochem. Biotech. 47: 157 - 173) to give a library of 6 x 10^7 clones.

4. <u>Large single chain Fv library</u> derived from lymphoid tissues including tonsil, bone marrow and peripheral blood lymphocytes.

Polyadenylated RNA was prepared from the B-cells of various lymphoid tissues of 43 non-immunised donors using the "Quickprep mRNA Kit" (Pharmacia). First-strand cDNA was synthesized from mRNA using a "First-strand cDNA synthesis" kit (Pharmacia) using random hexamers to prime synthesis. V-genes were amplified using family-specific primers for VH, Vκ and Vλ genes as previously described (Marks et al., supra) and subsequently recombined together with the (Gly_4,

Ser)$_3$ scFv linker by PCR assembly. The VH-linker-VL antibody constructs were cloned into the *Sfi* I and *Not* I sites of the phagemid vector, pCANTAB 6. Ligation, electroporation and plating out of the cells was as described previously (Marks et al, 1991 supra). The library was made ca. 1000x larger than that described previously by bulking up the amounts of vector and insert used and by performing multiple electroporations. This generated a scFv repertoire that was calculated to have ca. 1.3×10^{10} individual recombinants which by *Bst* NI fingerprinting were shown to be extremely diverse.

a. Induction of phage antibody libraries

The four different phage antibody repertoires above were selected for antibodies to TGFβ-1. The VH synthetic (Nissim et al., 1994 supra), tonsil, 'large' scFv and PBL (Marks et al., 1991 supra) repertoires were each treated as follows in order to rescue phagemid particles. 500 ml prewarmed (37 ^{0}C) 2YTAG (2YT media supplemented with 100 μg/ml ampicillin and 2 % glucose) in a 2 l conical flask was inoculated with approximately 3×10^{10} cells from a glycerol stock (-70 ^{0}C) culture of the appropriate library. The culture was grown at 37 ^{0}C with good aeration until the OD_{600nm} reached 0.7 (approximately 2 hours). M13K07 helper phage (Stratagene) was added to the culture to a multiplicity of infection (moi) of

approximately 10 (assuming that an OD_{600nm} of 1 is equivalent to 5 x 10^8 cells per ml of culture). The culture was incubated stationary at 37 ^{0}C for 15 minutes followed by 45 minutes with light aeration (200 rpm) at the same temperature. The culture was centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 500 ml 2YTAK (2YT media supplemented with 100 μg/ml ampicillin and 50 μg/ml kanamycin), and the culture incubated overnight at 30 ^{0}C with good aeration (300 rpm). Phage particles were purified and concentrated by three polyethylene glycol (PEG) precipitations (Sambrook, J., Fritsch, E.F., & Maniatis, T. (1990). Molecular Cloning - A Laboratory Manual. Cold Spring Harbour, New York) and resuspended in PBS to 10^{12} transducing units (tu)/ml (ampicillin resistant clones).

b. Panning of phage antibody library on TGFβ-1

Phage induced from the four repertoires were each separately panned on TGFβ-1. A 75mm x 12mm immuno tube (Nunc; Maxisorp) was coated with 2 ml of recombinant human TGFβ-1 (0.5ug/ml, Genzyme) in PBS overnight at 4 ^{0}C. After washing 3 times with PBS, the tube was filled with 3%MPBS (3 % 'Marvel' skimmed milk powder, 1x PBS) and incubated for 2 hours at 37 ^{0}C for blocking. The wash was repeated, phagemid particles (10^{13} tu) in 2 ml of 3% MPBS were added and the tube

incubated stationary at 37 ^{0}C for 1 hour. The tube was washed 20 times with PBST (0.1%), then 20 times with PBS. Bound phage particles were eluted from the tube by adding 2 ml of 100mM-triethylamine, and incubating the tube stationary at room temperature for 10 minutes. The eluted material was immediately neutralised by pipetting into a tube containing 1 ml 1M-Tris.HCl (pH7.4). Phage were stored at 4 ^{0}C. 1.5 ml of the eluted phage were used to infect 20 ml of logarithmically growing E. coli TG1 (Gibson, T.J. (1984). PhD thesis. University of Cambridge, UK.). Infected cells were grown for 1 hour at 37 ^{0}C with light aeration in 2YT broth, and then plated on 2YTAG medium in 243mm x 243mm dishes (Nunc). Plates were incubated overnight at 30 ^{0}C. Colonies were scraped off the plates into 10 ml of 2YT broth and 15 % (v/v) glycerol added for storage at -70 ^{0}C.

Glycerol stock cultures from the first round of panning of each of the four repertoires on TGFβ-1 were each rescued using helper phage to derive phagemid particles for the second round of panning. 250 μl of glycerol stock was used to inoculate 50 ml 2YTAG broth, and incubated in a 250 mL conical flask at 37 ^{0}C with good aeration until the OD_{600mn} reached 0.7 (approximately 2 hours). M13K07 helper phage (moi=10) was added to the culture which was then incubated stationary at 37 ^{0}C for 15 minutes followed by 45 minutes with light aeration (200 rpm) at the same

temperature. The culture was centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 50 ml prewarmed 2YTAK, and the culture incubated overnight at 30 ^{0}C with good aeration. Phage particles were purified and concentrated by PEG precipitation (Sambrook et al., 1990 supra) and resuspended in PBS to 1013 tu/ml.

Phage induced from the first round of panning of each of the three repertoires, was selected a second time essentially as described above except that the panning tube was coated with only 1 ml of TGFβ-1 (0.5ug/ml, Genzyme), and the volume of phage added to the tube similarly reduced. After extensive washing, bound phage were eluted from the tube using 1 ml of 100 mM-triethylamine, and neutralised by the addition of 0.5 ml 1M-Tris.HCl (pH7.4) as earlier described. The process of phage growth and panning was repeated over a third and a fourth round of selection.

c. Growth of single selected clones for immunoassay

Individual colonies from the third and fourth round selections were used to inoculate 100 μl 2YTAG into individual wells of 96 well tissue culture plates (Corning). Plates were incubated at 30 ^{0}C overnight with moderate shaking (200 rpm). Glycerol to 15 % was added to each well and these master plates stored at -70 ^{0}C until ready for analysis.

d. **ELISA to identify anti-TGFβ-1 scFv**

Clones specific for TGFβ-1 were identified by ELISA, using scFv displayed on phage or soluble scFv.

i. **Phage ELISA**

Cells from the master plates were used to inoculate fresh 96 well tissue culture plates containing 100 μl 2YTAG per well. These plates were incubated at 37 ^{0}C for 6-8 hours or until the cells in the wells were growing logarithmically (OD600 0.2-1.0). M13K07 was added to each well to an moi of 10 and incubated stationary for 15 min then 45 min with gentle shaking (100 rpm), both at 37 ^{0}C. The plates were centrifuged at 2000 rpm for 10 min and the supernatant eluted. Each cell pellet was resuspended in 100 μl 2YTAK and incubated at 30 ^{0}C overnight.

Each plate was centrifuged at 2000 rpm and the 100 μl supernatant from each well recovered and blocked in 20 μl 18%M6PBS (18 % skimmed milk powder, 6 x PBS), stationary at room temperature for 1 hour. Meanwhile, flexible microtitre plates which had been blocked overnight stationary at 4 ^{0}C with either 50 μl 0.2 μg/ml TGFβ-1 in PBS or 50 μl PBS alone (giving an uncoated control plate), were washed 3 times in PBS and blocked for 2 h stationary at 37 ^{0}C in 3MPBS. These plates were then washed three times with PBS and 50 μl preblocked phage added to each well of both the TGFβ-1-coated or uncoated plate. The plates were

incubated stationary at 37 ^{0}C for 1 h after which the phage were poured off. The plates were washed by incubating for 2 min in PBST three times followed by incubating for 2min in PBS three times, all at room temperature.

To each well of both the TGFβ-1-coated and the uncoated plate, 50 μl of a 1 in 10,000 dilution of sheep anti-fd antibody (Pharmacia) in 3MPBS was added and the plates incubated at 37 ^{0}C stationary for 1 h. Each plate was washed as described above and 50 μl of a 1 in 5,000 dilution donkey anti-sheep alkaline phosphatase conjugate (Sigma) in 3MPBS added and incubated stationary at 37 ^{0}C for 1 h. Plates were washed as described as above followed by two rinses in 0.9% NaCl. Alkaline phosphatase activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). The absorbance signal generated by each clone was assessed by measuring the optical density at either 405 nm (pNPP) or 492 nm (Ampak) using a microtitre plate reader. Clones were chosen for further analysis if the ELISA signal generated on the TGFβ-1-coated plate was at least double that on the uncoated plate.

ii. Soluble ELISA

Cells from the master plates were used to inoculate fresh 96 well tissue culture plates containing 100 μl 2YTAG per well. These plates were

incubated at 30 ^{0}C for 8 hours then centrifuged at 2000 rpm for 10 min and the supernatant eluted. Each cell pellet was resuspended in 100 μl 2YTA (2YT media supplemented with 100ug/ml ampicillin) containing 10 mM IPTG (isopropyl-B-D-thiogalactopyranoside) and incubated at 30 ^{0}C overnight.

Each plate was centrifuged at 2000 rpm and the 100 µl supernatant from each well recovered and blocked in 20 µl 18%M6PBS stationary at room temperature for 1 hour. Meanwhile, flexible microtitre plates which had been blocked overnight stationary at 4 ^{0}C with either 50 µl 0.2 µg/ml TGFβ-1 in PBS or 50 µl PBS alone, were washed 3 times in PBS and blocked for 2 h stationary at 37 ^{0}C in 3%MPBS. These plates were then washed three times with PBS and 50 µl preblocked soluble scFv added to each well of both the TGFβ-1-coated or uncoated plate. The plates were incubated stationary at 37 ^{0}C for 1 h after which the scFv solutions were poured off. The plates were washed by incubating for 2 min in PBST (PBS containing 1% Tween) three times followed by incubating for 2 min in PBS three times, all at room temperature.

To each well of both the TGFβ-1-coated and the uncoated plate, 50 µl of a 1 in 200 dilution of the anti-myc tag murine antibody 9E10 (Munro, S. & Pelham, H.R.B. (1986)Cell 46, 291-300) in 3MPBS was added and the plates incubated at 37 ^{0}C stationary for 1 h.

Each plate was washed as described above and 50 µl of a 1 in 5,000 dilution goat anti-mouse alkaline phosphatase conjugate (Pierce) in 3MPBS added and incubated stationary at 37 ^{0}C for 1 h. Plates were washed as described above followed by two rinses in 0.9% NaCl. Alkaline phosphatase activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). The absorbance signal generated by each clone was assessed by measuring the optical density at either 405 nm (pNPP) or 492 nm (Ampak) using a microtitre plate reader. Clones were chosen for further analysis if the ELISA signal generated on the TGFβ-1-coated plate was at least double that on the uncoated plate.

iii. Specificity ELISA

Clones identified as binding TGFβ-1 rather an uncoated well, as described above, were further analysed for fine specificity. Specificity ELISA's were carried out using scFv either displayed on phage or in solution as described above, except that 5 ml of media in 50 ml Falcon tubes were inoculated with each clone and grown to generate the phage or soluble scFv used in the ELISA. Microtitre plate wells were coated with 50 µl of either 0.2 µg/ml TGFβ-1, 0.2 µg/ml TGFβ-2, 10 µg/ml bovine serum albumin (BSA) or PBS (the uncoated well). After preblocking both the phage (or soluble scFv) and the microtitre plates, 50 µl

blocked phage (or soluble scFv) from each clone was added to a well coated with either TGFβ-1, TGFβ-2, BSA or an uncoated well. As above, alkaline phosphatse activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). Clones were considered to be specific for TGFβ-1 if the ELISA signal generated in the TGFβ-1 coated well was at least five-fold greater than the signal on either TGFβ-2, BSA or an uncoated well.

iv. Specificity determination by BIACore™

The antibodies were also shown to be specific for TGFβ1 compared to TGFβ2 (obtained from R&D Systems Abingdon) by relative binding to theBIACore™ sensor chips coated with the appropriate antigen. TGFβ1 and TGFβ2 were immobilised by amine coupling to Biosensor CM5 sensorchips (Pharmacia) according to the manufacturers instructions. Single chain Fv fragments (35μl; purified by immobilized metal affinity chromatography as described in example 4) were injected over the immobilized antigen at a flow rate of 5μl/min. The amount of TGFβ bound was assessed as the total increase in resonance units (RUs) over this period. For 31G9 scFv an increase of 1059RUs was found with a TGFβ1 chip and 72 RUs was found with a TGFβ2 chip. Thus binding is much stronger to TGFβ1 than TGFβ2.

e. Sequencing of TGFb1-Specific ScFv Antibodies

The nucleotide sequence of the TGFβ-1 specific antibodies was determined by first using vector-specific primers to amplify the inserted DNA from each clone. Cells from an individual colony on a 2YTAG agar plate were used as the template for a polymerase chain reaction (PCR) amplification of the inserted DNA using the primers pUC19reverse and fdtetseq (Table 1). Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 2 min, followed by 10 min at 72 ^{0}C. The PCR products were purified using a PCR Clean-up Kit (Promega) in to a final volume of 50 μl H20. Between 2 and 5 μl of each insert preparation was used as the template for sequencing using the Taq Dye-terminator cycle sequencing system (Applied Biosystems). The primers mycseq10 and PCR-L-Link were used to sequence the light chain of each clone and PCR-H-Link and pUC19reverse to sequence the heavy chain (Table 1)

f. Sequence and Source of the Initial TGFβ-1-Specific ScFv Antibodies

Four different TGFβ-1 specific antibodies were isolated from the selections using the four libraries described above. Each clone name, its origin and its heavy and light chain germline is given below. The complete sequence of each VH domain gene is given in

figure 1(a), together with the VL domain gene, from scFv 31G9.

CLONE	LIBRARY SOURCE	VH GERMLINE		VL ISOTYPE
1-B2	PBL	VH3	DP49	VKappa
1A-E5	Synthetic VH	VH3	DP53	VLambda
1A-H6	Tonsil	VH3	DP50	VLambda
31-G9	large scFv	VH3	DP49	VLambda

Thus these initial isolates were obtained from libraries derived from different sources-both natural V genes of unimmunised humans and synthetic libraries from cloned germline V genes together with synthetic CDRs.

2. Affinity Maturation of the Initial TGFβ-1-Specific ScFv Antibodies

a. Light Chain Shuffling of the TGFβ-1-Specific ScFv Antibody 1-B2

i. Construction of Repertoires

The heavy chain of clone 1-B2 was recombined with the complete repertoire of light chains derived from the PBL and large (tonsil-derived) scFv repertoires. The 1-B2 heavy chain was amplified by PCR using the primers HuJh4-5For (Table 1) and pUC19reverse.

Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VH excised, and eluted from the agarose gel using the Geneclean Kit (Bio 101).

The PBL and tonsil light chains were amplified by PCR using the primers fdtetseq and a mix of RL1, 2 & 3 (Table 1). Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VL excised, and eluted from the agarose gel using the Geneclean Kit (Bio 101).

Approximately 50 ng amplified 1-B2 heavy chain and 50 ng of either amplified PBL-derived or amplified tonsil-derived light chains were combined and precipitated with sodium acetate and ethanol using 25 μg glycogen as a carrier. The precipitated DNA was pelleted by centrifugation at 13,000 rpm in a microfuge, air dried and resuspended in 26 μl H20. This was used in an assembly amplification after the addition of reaction buffer to 1X, dNTP's to 200 nM and 5 units Taq polymerase. Amplification conditions consisted of 20 cycles of 94 ^{0}C for 1 min, 60 ^{0}C for 1 min and 72 ^{0}C for 1min 30 s, followed by 10 min at 72 ^{0}C. 10 μl of each assembly was used as the template

in a 'pull-through' amplification with the primers fdtetseq and pUC19reverse. Amplification conditions consisted of 25 cycles of 94 ^{0}C for 1 min, 60 ^{0}C for 1 min and 72 ^{0}C for 1min 30 s, followed by 10 min at 72 ^{0}C.

The pull-through amplification product was separated through 1% agarose-TAE and the band representing the pull-through VH-VL excised and eluted using the Geneclean Kit. This was digested with the restriction endonucleases Sfi I and Not I (NEB) and ligated (Amersham ligation system) into the phagemid vector pCantab 6, previously digested with Sfi 1 and Not I. The ligation product was used to transform electrocompetent TG1 cells, plated out on 2YTAG plates and incubated overnight at 30 ^{0}C. Approximately 1 x 10^5 individual clones were generated from the light chain-shuffle of the 1-B2 heavy chain with the PBL-derived light chains and approximately 1 x 10^6 for the shuffle with the tonsil-derived light chains.

ii. Selection of Light Chain Shuffle Repertoires

The two light chain-shuffle repertoires were selected for TGFβ-1-specific antibodies. Phagemid particles were recovered from each repertoire as described earlier for the initial libraries. Recovered phage were preblocked for 1 h in a final volume of 100 µl 3MPBS. Approximately 10^{11} tu phage were used in the first round selection and between 10^9

and 10^{10} for subsequent selections. For the first round selections, biotinylated TGFβ1 to a final concentration of 100 nM was added to the preblocked phage and incubated stationary at 37°C for 1h.

For each selection, 100 µl Dynabeads suspension (Dynal) was separated on a magnet and the beads recovered and preblocked for 2 h in 1 ml 3MPBS. The beads were recovered on a magnet and resuspended in the phagemid/biotinylated TGFβ-1 mixture and incubated at room temperature for 15 min while being turned end-over-end. The beads were captured on a magnet and washed four times with PBST followed by three washes in PBS. After each wash, the beads were captured on a magnet and resuspended in the next wash. Finally, half of the beads were resuspended in 10 µl 50 mM DTT (the other half of the beads stored at 4 °C as a back-up) and incubated at room temperature for 5 min. The whole bead suspension was then used to infect 5 ml logarithmically-growing TG1 cells. This was incubated at 37 °C, stationary for 15 min then with moderate shaking for 45 min, plated on 2YTAG plates and incubated overnight at 30 °C.

Colonies were scraped off the plates into 10 ml of 2YT broth and 15 % (v/v) glycerol added for storage at -70 °C. A 250 µl aliqout of each plate scrape was used to inoculate 2YTAG and phagemid particles rescued as described earlier. For each repertoire, three rounds of selection using biotinylated TGFβ-1 was

performed, essentially identical to the first round selection described above. All selections were at 100 nM TGFβ-1 except for the third round selection of the tonsil-derived light chain repertoire where the concentration of biotinylated TGFβ-1 in the selection was reduced to 50 nM.

iii. Identification of TGFβ-1-Specific ScFv Antibodies from Light Chain Shuffle Repertoires

ScFv antibodies specific to TGFβ-1 were identified by both phage and soluble ELISA, and sequenced, as described earlier. Three new TGFβ-1-specific scFv antibodies were identified, two with PBL-derived light chains and one with a tonsil-derived light chain. All three had the 1B2 heavy chain sequence (DP49), described earlier. The sequences are summarised below and the complete sequence of each VL domain gene is given in figure 1(b).

CLONE	VL SOURCE	VH GERMLINE	VL ISOTYPE
7-A3	PBL	DP49 (1B2)	VKappa
10-A6	PBL	DP49 (1B2)	VLambda
14-A1	Tonsil	DP49 (1B2)	VLambda

Thus the VH domain 1B2 derived from the PBL library can be combined with VL domains derived from

both PBL and tonsil libraries.

b. CDR3 'Spiking' of the TGFβ-1-Specific ScFv Antibody 1B2

i. Construction of 'spiked' repertoire

An 84 mer mutagenic oligonucleotide primer, 1B2 mutVHCDR3, was first synthesized (see Table 1). This primer was 'spiked' at 10%; i.e. at each nucleotide position there is a 10% probability that a non-parental nucleotide will be incorporated. The 1-B2 heavy chain was amplified by PCR using the primers pUC19reverse and 1B2 mutVHCDR3. Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VH excised, and eluted from the agarose gel using the Geneclean Kit (Bio 101).

The parental 1B2 light chain was amplified by PCR using the primers fdtetseq and RL3 (Table 1). Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VL excised, and eluted from the agarose gel using the Geneclean Kit (Bio 101).

Approximately 50 ng amplified 'spiked' 1-B2 heavy

chain and 50 ng of amplified parental 1B2 light chain were combined and precipitated with sodium acetate and ethanol using 25 µg glycogen as a carrier. The precipitated DNA was pelleted by centrifugation at 13,000 rpm in a microfuge, air dried and resuspended in 26 µl H20. This was used in an assembly amplification after the addition of reaction buffer to 1X, dNTP's to 200 nM and 5 units Taq polymerase. Amplification conditions consisted of 25 cycles of 94 ^{0}C for 1 min, 65 ^{0}C for 4 min. Five µl of each assembly was used as the template in a 'pull-through' amplification with the primers fdtetseq and pUC19reverse. Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 2 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C.

The pull-through amplification product was separated through 1% agarose-TAE and the band representing the pull-through 'spiked' VH -VL excised and eluted using the Geneclean Kit. This was digested with the restriction endonucleases Sfi I and Not I (NEB) and ligated (Amersham ligation system) into the phagemid vector pCantab 6, previously digested with Sfi I and Not I. The ligation product was used to transform electrocompetent TG1 cells, plated out on 2YTAG plates and incubated overnight at 30 ^{0}C. Approximately 4 x 10^6 individual clones were generated from this VH CDR3 'spiking' of the 1-B2 VH CDR3.

ii. Selection of 1B2 CDR3 Spike Repertoire

The repertoire was selected for new TGFβ-1-specific scFv antibody by one round of panning on 1 μg/ml TGFβ-1 followed by two rounds of selection with biotinylated TGFβ-1 at 50 nM using methods as described earlier.

iii. Identification of TGFβ-1-Specific ScFv Antibodies from the 1B2 CDR3 Spike Repertoire

ScFv antibodies specific to TGFβ-1 were identified by both phage and soluble and phage ELISA, and sequenced, as described earlier. Clone 27C1 was isolated from the spiked repertoire. It is virtually identical to clone 1B2 but with three differences in the heavy chain CDR3. The complete sequence of clone 27C1 is given in figure 1 (c). The 27C1 VH domain was combined with the 10A6 VL domain in the construction of the whole antibody 27C1/10A6 IgG4 (example 2). The properties of this antibody are described in more detail in examples 2 to 6. In addition to 27C1, a large number of other antibodies were isolated with up to 7 of the 14 amino acids differing in CDR3 of the VH domain (Figure 3). These had a similar preference for binding TGFβ1 compared to TGFβ2.

3. Identification and Characterisation of Antibodies to Human TGFβ-2 by Selection of Naive and Synthetic Phage Antibody Repertoires

a. Induction of phage antibody libraries

Two different phage antibody repertoires were selected for antibodies to TGFβ-2. The VH synthetic (Nissim et al., 1994) and tonsil (constructed as described earlier) repertoires were each treated as described for TGFβ-1 to rescue phagemid particles.

b. Panning of phage antibody library on TGFβ-2

Phage induced from the two repertoires were each separately panned on TGFβ-2 as described earlier for TGFβ-1 but using 0.5 μg/ml TGFβ-2 as the coating antigen.

c. Identification and Sequencing of TGFβ-2-Specific ScFv Antibodies

Individual colonies from the third and fourth round selections were screened by both phage and soluble ELISA as described earlier for TGFβ-1 but using flexible microtitre plates coated with TGFβ-2 at 0.2 μg/ml rather than TGFβ-1. Clones were chosen for further analysis if the ELISA signal generated on the TGFβ-2-coated plate was at least double that on the uncoated plate. For the specificity ELISA, as described earlier for TGFβ-1, clones were considered to be specific for TGFβ-2 if the ELISA signal generated in the TGFβ-2 coated well was at least five-fold greater than the signal on either TGFβ-1,

BSA or an uncoated well.

d. Sequence and Source of the Initial TGFβ-2-Specific ScFv Antibodies

Four different TGFβ-2 specific antibodies were isolated from the selections using the two libraries described above. Each clone name, its origin and its heavy and light chain germline is given below. The complete sequence of each VH domain gene is given in figure 2 (a) together with the VL domain of Gold-11.

CLONE	LIBRARY SOURCE	VH GERMLINE	VL ISOTYPE
1-G2	Tonsil		
1-H6	Tonsil	DP49	
2A-H11	Synthetic VH	DP50	VLambda
2A-A9	Synthetic	DP46	VLambda
Gold-11	Large scFv		VLambda

Thus human antibodies binding to human TGFβ2 have been isolated from different sources-, both natural Vgenes of unimmunised humans and synthetic libraries from cloned germline V genes together with synthetic CDRs.

4. Light Chain Shuffling of the TGFβ-2-Specific ScFv Antibodies 2A-H11 and 2A-A9

a. Construction of Repertoires

The heavy chain of clones 2A-H11 and 2A-A9 were recombined with the complete repertoire of light chains derived from the PBL and large (tonsil-derived) scFv repertoires as described earlier for the TGFβ-1-specific scFv antibody 1-B2. Both repertoires generated from the recombination with the PBL light chain repertoire were approximately 1 x 10^5, those generated from the recombination with the tonsil light chain repertoire were approximately 1 x 10^6.

b. Selection of Light Chain Shuffle Repertoires

The light chain-shuffle repertoires were selected for TGFβ-2-specific antibodies using biotinylated TGFβ-2, as described earlier for the selection of the TGFβ-1 light chain shuffle repertoires. For all of the first and second round selections, a concentrartion of 100 nM biotinylated TGFβ-2 was used. For the third round selection of the PBL-derived light chain shuffle repertoire, biotinylated TGFβ-2 was used at concentrations of 100 nM and 1 nM. For the third round selection of the tonsil-derived light chain shuffle repertoire, biotinylated TGFβ-2 was used at a concentration of 50 nM.

c. Identification of TGFβ-2-Specific ScFv Antibodies from Light Chain Shuffle Repertoires

ScFv antibodies specific to TGFβ-2 were

identified by both phage and soluble ELISA, and sequenced, as described earlier. Five new TGFβ-2-specific scFv antibodies were identified. The sequences are summarised below and the complete sequence of each clone given in figure 2 (b).

CLONE	VL SOURCE	VH GERMLINE	VL ISOTYPE
6-H1	PBL	DP50 (2A-H11)	VKappa
6-A5	PBL	DP50 (2A-H11)	VLambda
6-B1	PBL	DP50 (2A-H11)	VLambda
11-E6	PBL	DP46 (2A-A9)	VKappa
14-F12	Tonsil	DP46 (2A-A9)	VLambda

d. Specificity determination by ELISA

Clones identified as binding TGFβ-2 rather an uncoated well, as described above, were further analysed for fine specificity. Specificity ELISA's were carried out using scFv either displayed on phage or in solution as described above, except that 5 ml of media in 50 ml Falcon tubes were inoculated with each clone and grown to generate the phage or soluble scFv used in the ELISA. Microtitre plate wells were coated with 50 μl of either 0.2 μg/ml TGFβ-1, 0.2 μg/ml TGFβ-2, 10 μg/ml bovine serum albumin (BSA) or PBS (the uncoated well). After preblocking both the phage (or soluble scFv) and the microtitre plates, 50 μl blocked phage (or soluble scFv) from each clone was

added to a well coated with either TGFβ-1, TGFβ-2, BSA or an uncoated well. As above, alkaline phosphatse activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). Clones were considered to be specific for TGFβ-2 if the ELISA signal generated in the TGFβ-2 coated well was at least five-fold greater than the signal on either TGFβ-2, BSA or an uncoated well. Cross-reactivity with unrelated antigens was determined more extensively for anti-TGFβ2 antibody in whole antibody format, see example 2. The cross-reactivity of 6B1 IgG4 and 6A5 IgG4 with TGFβ1 and TGFβ3 (obtained from R&D Systems, Abingdon) is also shown to be very low.

e. Specificity determination by BIACore™

The antibodies were also shown to be specific for TGFβ2 compared to TGFβ1 by relative binding to theBIACore sensor chips coated with the appropriate antigen. TGFβ1 and TGFβ2 were immobilised by amine coupling to Biosensor CM5 sensorchips (Pharmacia) according to the manufacturers instructions. Single chain Fv fragments (35μl; purified by immobilized metal affinity chromatography) were injected over the immobilized antigen at a flow rate of 5μl/min. The amount of TGFβ bound was assessed as the total increase in resonance units (RUs) over this period. For the single chain Fv fragments 6H1, 6A5 and 14F12,

these fragments gave a total of 686, 480 and 616 RUs respectively for the TGFβ1 coated sensor chip and 77, 71 and 115 RUs respectively for the TGFβ2 coated chip.

5. Building higher affinity anti TGFβ-1 biological neutralisers

a. Recombining heavy chains derived from high affinity anti- TGFβ1 scFv with light chains derived from anti -TGFβ1 and anti-TGFβ2 scFv showing good properties

Antibodies derived by spiking CDR3 of the scFv antibody 1-B2 (section 2b) bind TGFβ-1 with high affinity. To improve the chance of obtaining high affinity neutralising antibodies it was decided to chain shuffle VHs derived from high affinity anti-TGFβ-1 scFv with VLs derived from scFv clones with promising properties and particularly with those capable of neutralising the activity of TGFβ-2 in vitro.

Heavy chains were amplified by PCR from the repertoire of CDR3 spiked 1-B2 clones after selection on TGFβ-1(section 2a.ii) using the primers pUC19reverse and PCR-H-Link (Table 1). Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VH excised, and eluted from the agarose gel using the

Geneclean Kit (Bio 101).

Light chains were separately amplified by PCR from each of the anti TGFβ-1 specific neutralisers (7-A3, 10-A6 and 14-A1; section 2a.iii) and each of the anti TGFβ-2 specific neutralisers (6H1, 6A5, 6B1, 11E6 and 14F12; section 4c) using the primers fdtetseq1 and PCR-L-Link (Table 1). The same PCR conditions were used as described for theVH amplification. Each VL PCR product was then separately purified through a 1% agarose-TAE gel as described above. Purified products were finally mixed in approximately equimolar amounts (as estimated from an analytical agarose gel) to provide a VL 'pool'.

Approximately 50 ng amplified heavy chains and 50 ng of amplified pooled light chains were combined and precipitated with sodium acetate and ethanol using 25 µg glycogen as a carrier. The precipitated DNA was pelleted by centrifugation at 13,000 rpm in a microfuge, air dried and resuspended in 23 µl H20. This was used in an assembly amplification after the addition of reaction buffer, dNTP's to 200 nM and 5 units Taq polymerase. Amplification conditions consisted of 20 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 2 mins, followed by 10 min at 72 ^{0}C. 5 µl of assembly was used as the template in a 50ul 'pull-through' amplification with the primers fdtetseq and pUC19reverse. Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72

^{0}C for 2mins, followed by 10 min at 72 ^{0}C.

The pull-through amplification product was separated through 1% agarose-TAE and the band representing the pull-through VH-VL excised and eluted using the Geneclean Kit. This was digested with the restriction endonucleases Sfi I and Not I (NEB) and ligated into the phagemid vector pCantab 6 (McCafferty et al. 1994 supra), previously digested with Sfi 1 and Not I, using the Amersham ligation system. The ligation product was used to transform electrocompetent TG1 cells, plated out on 2YTAG plates and incubated overnight at 30 ^{0}C. A repertoire of approximately 3 x 10^6 individual clones was generated.

b. Selection of chain shuffled repertoire

The chain shuffled repertoire was selected by a single round of panning on TGFβ-1 (1ug/ml), as previously described (section 1b).

c. Identification of TGFβ-1 specific scFv antibodies

ScFv antibodies specific to TGFβ-1 were identified by phage ELISA and sequenced as described earlier (sections 1d.i andle). New TGFβ-1 specific scFv antibodies were identified. Two new high affinity clones were isolated -CS32 which consists of 31G9 VH and 7A3 VL and CS39 which consists of 31G9 VH and 6H1 VL.

d. Off-rate determination for single chain Fv fragments binding to TGFβ1 and TGFβ2

The off-rates for binding to TGFβ1 or TGFβ2 of the single chain Fv fragments described in this example were determined as described by Karlsson et al (R. Karlsson et al, J. Immunol. Methods 145, 229-240, 1991). The results obtained are shown in Table 2, together with dissociation constants for those which have been determined. These results indicate that high affinity antibodies have been isolated.

6. Identification and Characterisation of an Antibody which Cross-reacts with both Human TGFβ-1 and TGFβ-2 but not TGFβ-3 by Selection of a Large ScFv Repertoire

a. Panning of the Library and Identification of Binders

The large scFv library (described earlier) was induced, phagemid particles rescued and panned as described earlier with the following modifications. For the first round of panning, 1012 tu library phage in 0.5 ml PBS were used (rather than the standard 2 ml), for the second round, 3.5×10^9 phage in 0.5 ml PBS were used. The immuno tube was coated with 10 μg TGFβ-2 in 0.5 ml PBS for both the first and second round of selection. Individual colonies from the second selection were screened by ELISA using 0.2 μg/ml TGFβ-1. Clones binding TGFβ-1 were further

screened on TGFβ-2, TGFβ-3, BSA and PBS. Clones were considered to be specific for both TGFβ-1 and TGFβ-2 if the ELISA signal generated in the TGFβ-1 and the TGFβ-2 coated wells were both at least five-fold greater than the signal on TGFβ-3, BSA and an uncoated well.

c. Identification of a TGFβ-1/TGFβ-2 Cross-reactive ScFv Antibody

A single scFv antibody specific for both TGFβ-1 and TGFβ-2 was identified by both phage and soluble ELISA, and sequenced, as described earlier. The complete sequence of the VL domain of the antibody gene VT37 is given in figure 4. The dissociation constant of this single chain Fv antibody was estimated by analysis using BIACore™ to be 4nM for TGFβ1 and 7nM for TGFβ2. Cross-reactivity for TGFβ3 was also determined. Purified VT37scFv at 8.3æg/ml was passed over BIACore™ sensor chips coated with TGFβ1 (500RUs coated); TGFβ2 (450RUs coated) or TGFβ3 (5500RUs coated). The relative response for VT37 scFv binding was: TGFβ1 - 391RU bound; TGFβ2 - 261RU bound or TGFβ3 - 24RU bound. Thus this antibody binds strongly to TGFβ1 and TGFβ2 but binding to TGF β 3 is not detectable above background.

Example 2 Construction of Cell Lines Expressing Whole Antibodies

For the construction of cell lines expressing IgG4 antibodies, variable domains were cloned into vectors expressing the human gamma 4 constant region for the VH domains or the human kappa or lambda constant regions for the VL domains.

To construct the whole antibody, 27C1/10A6 IgG4 (specific for $TGF\beta_1$), 27C1 VH DNA was prepared from the clone isolated above, in example 1. The VH gene was amplified by PCR using the oligonucleotides VH3BackSfiEu and VHJH6ForBam (Table 1) with cycles of 1 min at 94°C, 1 min at 55°C, 1.5 min at 72°C. Following digestion with SfiI and BamHI, the VH gene was cloned into the vector vhcassette2 (Figure 5) digested with SfiI and BamHI. Ligated DNA was transformed into E. coli TG1. Ampicillin resistant colonies were obtained and those containing the correct insert identified by DNA sequencing.

Plasmid DNA from these colonies was prepared and the DNA digested with HindIII and BamHI. The HindIII-BamHI restriction fragment was ligated into the human IgG4 heavy chain expression vector pG4D100 (Figure 6), which had been digested with HindIII and BamHI and the DNA transfected into E.*coli* TG1 by electroporation. The sequence of the VH gene insert was again verified by DNA sequencing.

For the light chain, the VL gene of 10A6, isolated in example 1, was first mutagenized to remove its internal BamHI site using site directed

mutagenesis (Amersham RPN1523) with the oligonucleotide DeltaBamHI (Table 1). The resulting VLDBamH1 gene was amplified by PCR using the oligonucleotides Vλ3/4BackEuApa and HuJλ2-3ForEuBam (Table 1). Following digestion of the amplified insert with ApaLI and BamHI, the VL gene was cloned into the vector vlcassetteCAT1 (Figure 7) digested with ApaLI and BamHI. Ligated DNA was transformed into E.coli TG1. Ampicillin resistant colonies were obtained and those containing the correct insert were identified by DNA sequencing.

Plasmid DNA from these colonies was prepared and the DNA digested with Hind III and BamHI. The HindIII-BamHI restriction fragment containing the leader sequence and the VL domain was ligated into the human lambda light chain expression vector, pLN10 (Figure 8), which had been digested with HindIII and BamHI. Following electroporation, transformants in E.coli were checked by DNA sequencing.

Plasmid DNA was prepared from the pG4D100-27C1 clone and the pLN10-10A6 clone. This DNA was then co-transfected into DUKXB11 Chinese Hamster Ovary (CHO) cells by electroporation (290V; 960μF). The cells were then grown for 2 days in non-selective medium (alpha-MEM plus nucleosides). Cells were then transferred to a selective medium (alpha-MEM plus 1mg/ml G418 without nucleosides) and grown in 96 well plates. Colonies were then transferred to 24 well

plates and samples assayed by sandwich ELISA for assembled human IgG4 antibody and by binding to TGFβ1 in ELISA (as in example 1). For the sandwich ELISA, goat anti-human IgG coated on to the ELISA plate and captured human IgG4 detected using goat antihuman lambda light chain alkaline phosphatase conjugate. High expressing cell lines were then derived by amplification of the inserted genes using selection in the presence of methotrexate (R.J. Kaufman Methods Enzymol. 185 537-566, 1990).

The whole antibody 6H1 IgG4 (specific for TGFβ2) was constructed in a similar way to the above construction of 27C1/10A6 IgG4. The 6H1 VH gene (example 2) was cloned into pG4D100 as for 27C1 above except that PCR amplification was performed with the oligonucleotides VH3BackSfiEu and VHJH1-2FORBam. The 6H1 VL gene (example 2) was subcloned into vlcassetteCAT1 as above except that PCR amplification was performed with the oligonucleotides Vk2BackEuApa and HuJk3FOREuBam. However, since the 6H1 VL is a kappa light chain the HindIII-BamHI fragment was subcloned into the human kappa light chain expression vector pKN100 (Figure 9) which had been digested with HindIII and BamHI. High expressing cell lines were then isolated as described above. Clones expressing antibody were identified from culture plates by sandwich ELISA for assembled human IgG4 antibody (detected using goat anti-human kappa light chain

conjugate and by binding to TGFβ2 in ELISA (as in example 2).

To construct the whole antibodies 6A5 IgG4 and 6B1 IgG4, the same 6H1 VH construct in pG4D100 was used as for 6H1IgG4 since these antibodies all have the same VH gene. The 6B1 and 6A5 genes were each subcloned into vlcassetteCAT1 as above for the 10A6 light chain except that PCR amplification was performed with the nucleotides Vλ3backEuApa and HuJλ2-3ForEuBam. The HindIII-BamHI restriction fragment was then subcloned into pLN10 as above. Clones expressing antibody were identified from culture plates by sandwich ELISA for assembled human IgG4 antibody (detected using goat anti-human kappa light chain conjugate and by binding to TGFβ2 in ELISA (as in example 2).

Properties of whole antibody constructs

Purification of whole antibodies

Serum-free supernatant from CHO cells producing the relevant IgG was clarified by centrifugation at 8000 rpm (Beckman JS2-21) prior to purification. The supernatant was applied to a HiTrap Protein A Sepharose prepacked affinity column from Pharmacia, either 1 or 5ml size, with binding capacities of 25 or 120 mg respectively. Each IgG had a dedicated column to avoid any potential carry over of material from one

purification to another. The column was equilibrated to phosphate buffered saline (PBS) with ten column volumes of 1xPBS prior to applying the supernatant. When all the supernatant had been applied to the column at a flow rate of 2-4 ml/minute, again, depending on the column size, the column was washed with ten column volumes of 1xPBS to remove any non-specifically bound material. Elution of the bound protein was achieved using 0.1M sodium acetate, adjusted to pH 3.3 with glacial acetic acid. The eluted material was collected in 8 fractions of 1.5 ml volume, and the amount of protein determined by measuring the absorbance at 280nm, and multiplying this value by 0.7 to get a value in mg/ml. This was then neutralised with 0.5ml of 1M Tris.HCl pH 9.0 per 1.5ml fraction, and the protein-containing fractions pooled and dialysed against 1x PBS to buffer exchange the IgG. The column was returned to neutral pH by running ten column volumes of 1xPBS through, and was stored in 20% ethanol as a preservative until required again.

A sample was then run on 10-15% SDS-PAGE (Phast system, Pharmacia) and silver stained. this allowed an assessment of the purity of the IgG preparation. This was usually found to be about 80-90%, with only a couple of other bands prominent on the stained gel.

Binding specificity by ELISA

The IgG4 antibodies 6B1 and 6A5 were shown to bind TGFβ2 with very low cross-reactivity to TGFβ1 and TGFβ3 and no detectable cross-reactivity with a range of non-specific antigens: interleukin-1; human lymphotoxin (TNFb); human insulin; human serum albumin; single stranded DNA; oxazolone-bovine serum albumin; keyhole limpet haemocyanin; chicken egg white trypsin inhibitor; chymotrypsinogen; cytochrome c; glyceraldehyde phosphate dehydrogenase; ovalbumin; hen egg lysozyme; bovine serum albumin and tumour necrosis factor a - (TNF*a*) (Figure 13(a) and (b)). Likewise the antibodies 6B1, 6A5 and 6H1 IgG4 bound strongly to TGFβ2 coated on a BIACore™ sensor chip but not significantly to TGFβ1 or TGFβ3 coated chips.

Binding properties of whole antibodies by BIACore™

The affinity constants of the above antibodies were determined by BIACore™, using the method of Karlsson et al. J. Immunol. Methods 145, 299-240, 1991 (supra) and found to be approximately 5nM for 27C1/10A6 IgG4 for TGFβ1 and 2nM for 6H1 IgG4 for TGFβ2. The antibody 27C1/10A6 IgG4 also shows some cross-reactivity with TGFβ2 coated onto Biosensor chips but the dissociation constant is approximately 10 fold or more higher for TGFβ2 compared to TGFβ1. There was no significant cross-reactivity with lysozyme coated onto a BIACore™ sensor chip.

Neutralisation and inhibition of radioreceptor

binding by IgG4 antibodies to TGFβ1 and TGFβ 2 is described in examples 3 and 4.

Example 3 Neutralisation by Antibodies of the Inhibitory Effect of TGF b1 and TGF b2 on Cell Proliferation

The neutralising activity of the antibodies described in examples 1 and 2 were tested in a modification of a bioassay for TGF β as described by Randall et al (1993) J. Immunol Methods 164, 61-67. This assay is based on the ability of TGF β_1 and TGF β_2 to inhibit the interleukin-5 induced proliferation of the erythroleukaemia cell line, TF1 and being able to reverse this inhibition with specific TGF β antibodies.

Method

Cells and maintenance

The human erythroleukaemia cell line TF1 was grown in RPMI 1640 medium supplemented with 5% foetal calf serum, penicillin/streptomycin and 2ng/ml rhGM-CSF in a humidified incubator containing 5% CO_2 at 37°C. Cultures were passaged when they reached a density of 2 X 10^5/ml and diluted to a density of 5 x 10^5/ml.

Cytokines and Antibodies

rhGM-CSF and rhIL-5 were obtained from R&D systems, rhTGF β_2 was obtained AMS Biotechnology. Rabbit anti TGF β_2 antibody was from R&D Systems and Mouse anti-TGF $\beta_{1,2,3}$ was from Genzyme. Other antibodies against TGF β_2 were as described in examples 1&2.

Titration of Inhibition of Proliferation by TGF β_2.

Doubling dilutions of TGF β_2 (800pM - 25pM) for the construction of a dose response curve were prepared on a sterile microtitre plate in 100µl of RPMI 1640 medium containing 5% foetal calf serum and antibiotics. All dilutions were performed at least in quadruplicate. Additional wells containing 100µl of the above medium for reagent and cells only controls were also included.

TF1 cells were washed twice in serum free RPMI 1640 medium and resuspended in RPMI 1640 medium supplemented with 5% foetal calf serum, 100U/ml penicillin and 100µg/ml streptomycin and 4ng/ml rhIL-5 at a density of 2.5 x 10^5/ml. Aliquots of 100µl were added to the previously prepared dilution series and the plate incubated for 48hr. in a humidified incubator containing 5% CO_2 at 37°C.

Cell proliferation was measured colourimetrically by addition of 40µl CellTiter96 substrate (Promega), returning the plate to the incubator for a further 4hr and finally determining the absorbance at 490nm. The

percentage inhibition for each concentration of TGF β_2 as compared to cell only wells was then calculated.

<u>Assay for Neutralisation of TGF β_2 Inhibitory Activity by Anti-TGF β_2 Antibodies</u>

Neutralisation of TGF β_2 was determined by making doubling dilutions in of each purified antibody in 100µl of medium as above. TGF β_2 was added to each antibody dilution to give a final concentration equivalent to that which gave 50% inhibition in the titration described above. Each dilution was prepared in quadruplicate. Additional wells were prepared for antibody only, cells only and reagent controls. Cell preparation and determination of cell proliferation was performed as described above.

<u>Results</u>

TGF β_2 was shown to inhibit the proliferation of TF1 cells by 50% at a concentration of 50pM. This concentration was used for all neutralisation experiments.

These assays showed that TGF β_2 activity was neutralised in a dose dependant manner for both scFv fragments (figure 10) and for whole IgG4 antibodies (figure 11). The concentration of antibody which gave 50% inhibition was determined from the graphs and is shown in table 4.

Example 4 Inhibition by antibodies of TGFβ binding to receptors measured in a radioreceptor assay

Single chain Fv fragments and whole IgG4 antibodies from different clones were expressed and purified and their ability to inhibit binding of TGFβ to receptors measured in a radioreceptor assay.

PURIFICATION OF scFv

ScFvs containing a poly histidine tail are purified by immobilised metal affinity chromatography. The bacterial clone containing the appropriate plasmid is inoculated into 50 ml 2TY medium containing 2% glucose and 100 μg/ml ampicillin (2TYAG) and grown overnight at 30°C. The next day the culture is added to 500 ml prewarmed 2TYAG and grown at 30°C for 1 h. The cells are collected by centrifugation and added to 500 ml 2TY containing ampicillin and 1 mM IPTG and grown at 30°C for 4 h. The cells are then collected by centrifugation and are resuspended in 30 ml ice-cold 50 mM Tris HCl pH 8.0, 20% (w/v) sucrose, 1 mM EDTA. After 15 min end-to-end mixing at 4°C the mixture is centrifuged at 12 k rpm for 15 min at 4°C. The supernatant is removed and to it added ~ 1ml NTA-agarose (Qiagen 30210) and mixed at 4°C for 30 min. The agarose beads are washed extensively with 50 mM sodium phosphate, 300 mM NaCl and loaded into a small column. After further washing with 50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole pH 7.4 scFv is

eluted with 50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole pH 7.4. 0.5 ml fractions are collected and the protein containing fractions identified by measuring the A_{280nm}. Pooled fractions are concentrated and scFv further purified by gel filtration in PBS on a Superdex 75 column (Pharmacia).

PURIFICATION OF WHOLE ANTIBODIES

Whole IgG4 antibodies were purified as described in example 2.

RADIORECEPTOR ASSAY FOR TGF-β

Neutralisation of TGF-β activity is measured by the ability of the scFvs and IgGs to inhibit the binding of ^{125}-I labelled TGF-β to its receptors on A549 human lung carcinoma cells.

A549 cells (ATCC CCL 185) are grown in high glucose Dulbecco's modified Eagle's medium (Sigma D-6546) supplemented with 10% foetal calf serum (PAA), 2 mM glutamine (Sigma G-7513), penicillin/streptomycin (Sigma P-0781), MEM non-essential amino acids (Sigma M-7145).

Cells are seeded at 1-2 x 105 cells / ml / well into the wells of 24-well cluster plates and grown for 24 h in serum-free DMEM. Cell monlayers are washed twice with serum-free DMEM and 0.5 ml binding medium (DMEM/Hams F12 (Sigma D-6421) containing 0.1% (v/v) BSA added to each well.

Aliqouts of ^{125}I-TGF-β1 or -β2 (70-90 TBq/mmol; Amersham International) at 20 pM are preincubated with antibody in binding medium at room temperature for 1 h. Duplicate samples of 0.5 ml of TGF-β/antibody mixtures are then added to the cell monlayers and are incubated at 37°C for 1-2 h. Control wells contain TGF-β only. Unbound TGF-β is removed by washing 4 times with Hank's balanced salt solution containing 0.1% BSA. Cells are solubilised in 0.8 ml 25 mM Tris HCl pH 7.5, 10 % glycerol, 1 % Triton X-100 at room temperature for 20 min. The contents of each well are removed and ^{125}I measured in a gamma counter. The potency of each scFv or IgG is measured by the concentration of antibody combining sites necessary to inhibit binding of TGF-β by 50% (IC50; Table 5). Thus the IC50 values are below 10nM and in some cases below 1nM indicating very potent antibodies.

Example 5 Prevention of Scar Formation by Antibodies Against TGF b1 and TGF b2 in the Injured Central Nervous System of the Rat

Logan *et al* (1994) Eur.3 Neuroscience 6,355-363 showed in a rat model of CNS injury, the ameliorating effect of a neutralising turkey antiserum directed against TGF β_1 on the deposition of fibrous scar tissue and the formation of a limiting glial membrane that borders the lesion. A study was set up to investigate the effects of neutralising engineered

human antibodies directed against both TGF β_1 and TGF β_2 in the same rat model. The derivation of the antibodies used in this study is described in examples 1 and 2.

Method

Animals and surgery

Groups of five female Sprague-Dawley rats (250g) were anaesthetised with an i.p. injection. The anaesthetised rats had a stereotactically defined lesion made into the right occipital cortex (Logan *et al* 1992 Brain Res. 587, P216-227) and the lateral ventricle was surgically cannulated and exteriorised at the same time (Logan *et al* 1994 supra).

Neutralisation of TGF β

Animals were intraventricularly injected daily with 5ul of purified anti TGF b antibodies (Table 3) diluted in a vehicle of artificial cerebrospinal fluid as described by Logan *et al* 1994 supra. Fourteen days post lesion all animals were perfusion fixed and 7mm polyester wax sections were processed for histochemical evaluation of the lesion site by immunofluorescent staining.

Fluorescent immunohistochemistry and image analysis

Morphological changes within the wound site were

followed by immunofluorescent staining with antibodies to fibronectin and laminin detected with anti-species FITC conjugates (Logan *et al* 1994 supra). These changes were semi-quantitatively assessed by image analysis using a Leitz confocal microscope linked to a Biorad MRC500 laser scanning system. Readings were taken at standard positions mid-way along the lesion.

Results

Effects of antibodies to TGF β at the site of CNS injury

Quantitation of the specific relative fluorescence for each of the antibodies is shown in figure 12 a and b. Laminin is a measure of the formation of the glial limitans externa along the boundaries of the wound and together with fibronectin forms a matrix of fibrous tissue within the centre of the wound. Quantitation by image analysis of these two proteins allows the degree of scarring at the wound site to be determined.

Compared with the saline control (fig.12 a,b), There is a considerable decrease in fibronectin and laminin immuno-localisation in the wound in the anti-TGF β antibody treated brains. Thus this indicates that these engineered human antibodies directed against epitopes on TGF β_1 & TGF β_2 ameliorate the effects of injury to the CNS both separately and

together. by preventing the deposition of the cellular matrix proteins fibronectin and laminin within the wound site. Previously Logan *et al* (1994 supra) had shown the effectiveness of a polyclonal turkey anti-sera directed against TGF β_1. This is the first report of any antibodies directed against TGF β_2 having been shown to be effective in this model.

Example 6 Suppression of experimental glomerulonephritis using human antibodies against human TGFβ

The ability of human antibodies against human TGFβ to neutralise TGFβ activity, and thus prove beneficial in the treatment of fibrotic disease, was tested in an animal model of the kidney disease, glomerulonephritis.

Antibodies directed against TGFβ1 have been shown to be effective in the suppression of experimental glomerulonephritis (W.A. Border et al Nature 346 371-374,1990) and other fibrotic diseases (W.A. Border & N.A. Noble New Engl. J. Med. 331 1286-1292, 1994). In this example, it is shown that antibodies directed against either TGFβ_1 or TGFβ_2 are effective in the treatment of glomerulonephritis. Induction of glomerulonephritis in rats with a single injection of anti-thymocyte serum was followed by treatment with an injection of either antibody directed against TGFβ_1 or of saline.

31G9 and 6A5 scFv (example 1) were expressed using a T7 polymerase controlled vector system (J.H. Christensen et al FEBS Lett. 281 181-184, 1991). Active scFv protein was prepared from inclusion bodies using the methodology described in WO94/18227 (H.C. Thøgersen et al). The scFv preparations were homogeneous as determined by SDS-PAGE and by gel filtration chromatography on Superose 12.

Five groups of rats were used-

Group A: Normal controls, no anti-thymocyte serum treatment

Group B: Disease control (saline treatment)

Group C: Treatment daily with 25μg 31G9 single chain Fv (anti-TGFβ_1)

Group D: Treatment daily with 25μg 6A5 single chain Fv (anti-TGFβ_2)

Group E: Treatment daily with 25μg 31G9 and 8æg 6A5 single chain Fv

Groups B to E each received a dose of 0.25ml sheep anti-thymocyte serum (ATS; Border et al, 1990 supra). One hour after ATS injection, each group received 200μl PBS (group B) or the appropriate antibody (200μl in PBS). On days 1 to 5, these doses were repeated for groups B to E. On day 6, all rats were sacrificed.

Urinary protein was measured (a measure of glomerular injury: J.M. Ginsberg et al New Engl. J.

Med. 309 1543-1550, 1983) for 24h on days 5 to 6 and was found to be significantly lower for the rats treated with 6A5 scFv than for the disease control (see Figure 14). The extent of glomerular injury was determined by examination of glomeruli stained with periodic acid-Schiff's base (30 glomeruli for each rat). These glomeruli are scored for the extent of glomerular matrix accumulation (30 glomeruli for each rat) on histological examination of stained sections (Border et al, 1990 supra; W.A. Border et al Nature 360 361-364, 1992). Scoring was performed by two independent scientists for each rat. There was a significantly lower increase in extracellular matrix deposition for the 6A5 scFv treated rat compared to the disease control (Figure 15). There was also a somewhat lower increase for 31G9 scFv but this difference in deposition was not statistically significant.

Hence the human antibody against human TGFβ_2 is effective in suppression of experimental glomerulonephritis.

<u>Example 7 Neutralisation by antibodies directed against TGFβ2 of the inhibitory effect of TGFβ isoforms on cell proliferation</u>

The neutralising activity of 6B1 IgG4, 6H1 IgG4 (purified as in example 2) and a mouse monoclonal antibody (Genzyme; J.R. Dasch et al., supra) was

measured for each of the TGFβ isoforms, TGFβ1, TGFβ2 and TGFβ3 in the TF1 cell proliferation assay described in Example 3. The concentration of TGFβ isoform was 100pM in each assay.

As shown in Figure 16, 6B1 IgG4 strongly neutralises TGFβ2 with an IC_{50} of approximately 2nM (Table 6). This compares to 10nM for the mouse monoclonal from Genzyme and 12nM for 6H1 IgG4. Neither 6B1 IgG4 nor 6H1 IgG4 significantly neutralise TGFβ1 (Fig. 17). However, there is significant neutralisation of TGFβ3 by both 6B1 (IC_{50} ca. 11nM) and 6H1 IgG4 ca. 20nM; Fig. 18). This is considerably less than the neutralisation potency of the Genzyme monoclonal (IC_{50} ca. 0.1nM).

Both 6B1 IgG4 and 6H1 IgG4 are stronger neutralisers of TGFβ2 activity than of TGFgβ3 activity. The neutralisation of TGFβ3 activity is greater than would be predicted from the relative binding of these two isoforms by the antibodies (example 2) and the relative binding in a radioreceptor assay (example 8).

<u>Example 8 Inhibition by antibodies directed against TGFβ2 of binding of other TGFβ isoforms to receptors measured in a radioreceptor assay</u>

The ability of 6B1 IgG4 to inhibit binding of TGFβ isoforms to receptors was measured in a radioreceptor assay as described in example 4.

6B1 IgG4 inhibited binding of ^{125}I-TGFβ2 with an IC_{50} of 0.05nM. There was no significant inhibition of binding of ^{125}I-TGFβ1 whereas for ^{125}I-TGFβ3 6B1 IgG4 inhibited binding with an IC_{50} of approximately 4nM (Table 6). This indicates the potency of 6B1 IgG4 in this assay and its selectivity for the neutralisation of TGFβ2 activity. Cross-reactivity with TGFβ3 in this assay is less than 2%.

Thus 6B1 IgG4 preferentially inhibits the binding of TGFβ2 to its receptors compared with binding of TGFβ3.

Table 1: Oligonucleotide primers used in the identification and characterisation of TGF-b1 antibodies.

Primer	Nucleotide sequence 5' to 3'
1B2 mutVHCDR3	5' CGT GGT CCC TTT GCC CCA GAC GTC CAC ACC ACT AGA ATC GTA GCC ACT ATA TTC CCC AGT TCG CGC ACA GTA ATA CAC AGC CGT
pUC19reverse	5' AGC GGA TAA CAA TTT CAC ACA GG 3'
fdtet seq	5' GTC GTC TTT CCA GAC GTT AGT 3'
PCR-H-Link	5' ACC GCC AGA GCC ACC TCC GCC 3'
PCR-L-Link	5' GGC GGA GGT GGC TCT GGC GGT 3'
myc seq 10	5' CTC TTC TGA GAT GAG TTT TTG 3'
HuJH4-5For	5' TGA GGA GAC GGT GAC CAG GGT TCC 3'
RL1	5' G(C/A)A CCC TGG TCA CCG TCT CCT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'
RL2	5' GGA CAA TGG TCA CCG TCT CTT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'
RL3	5' GGA CCA CGG TCA CCG TCT CCT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'

VH1b/7a back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (AG)TG CAG CTG GTG CA(AG) TCT GG-3'

VH1c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC (GC)AG GTC CAG CTG GT(AG) CAG TCT GG-3'

VH2b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (AG)TC ACC TTG AAG GAG TCT GG-3'

VH 3b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC (GC)AG GTG CAG CTG GTG GAG TCT GG-3'

VH3c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG (AT)C(TC) GG-3'

VH4b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3'

VH4c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (GC)TG CAG CTG CAG GAG TC(GC) GG-3'

VH5b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GA(AG) GTG CAG CTG GTG CAG TCT GG-3'

VH 6a back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

VH3BACKSfiEu 5'- AGC TCG GTC CTC GCA ACT GCG GCC CCT GGG GCC CAC AGC GAG GTG CAG CTG GTG GAG TCT GG - 3'

VHJH6FORBam 5'-CGA GTC ATT CTG CAC TTG GAT CCA CTC ACC TGA GGA GAC GGT GAC CGT GGT CCC - 3'

DeltaBamHI 5'-GA GAA TCG GTC TGG GAT TCC TGA GGG CCG G-3'

Vλ3/4BackEuApa 5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC CAC GTT ATA CTG ACT CAG GAC CC -3'

HuJλ2-3ForEuBam 5'-G GTC CTC GCA ACT GCG GAT CCA CTC ACC TAG GAC GGT CAG CTT GGT CCC- 3'

VHJH1-2FORBam 5'-CGA GTC ATT CTG CAC TTG GAT CCA CTC ACC TGA GGA GAC GGT GAC CAG GGT GCC - 3'

Vκ2BackEuApa 5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC GAT GTT GTG ATG ACT CAG TCT CC-3'

HuJκForEuBam 5'-G GTC CTC GCA ACT GCG GAT CCA CTC ACG TTT GAT ATC CAC TTT GGT CCC -3'

Vλ3BackEuApa 5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC TCG TCT GAG CTG ACT CAG GAC CC -3'

Table 2 Properties of single chain Fv fragments for binding to TGFbeta1 or TGFbeta2 determined using BIACore

Antibody	*koff* (s^{-1})	$K_d(nM)$
TGFbeta1		
31G9	9.0×10^{-4}	12
CS32	1.2×10^{-3}	
CS39	1.7×10^{-3}	
TGFbeta2		
6A5	1.4×10^{-4}	0.7
6B1	6.0×10^{-4}	
6H1	1.1×10^{-3}	
14F12	2.1×10^{-3}	

Table 3 Daily dose levels for individual animals in each group

Group	Clone	Antibody format	Antigen	Dose
1	Saline Control	-	-	-
2	31G9	scFv	TGF β_1	20ng
3	6A5	scFv	TGF β_2	20ng
4	27C1/10A6	IgG4	TGF β_1	692ng
5	6H1	IgG4	TGF β_2	1.76μg
6	31G9 +6A5	scFv's	TGF β_1 TGF β_2	20ng "
7	27C1/10A6 + 6H1	IgG4's	TGF β_1 TGF β_2	692ng 1.76μg

Table 4 I.C.$_{50}$ values for antibodies in TF1 assay

Antibody	scFv (nM)	IgG4 (nM)
6H1	1.5	100
6B1	15	11
6A5	8	150
14F12	90	nd

nd = not determined

Table 5 IC_{50} values for antibodies measured using a radioreceptor assay.

Anti-TGF-β1 antibody	IC_{50}, nM
7A3 scFv	>100
31G9 scFv	30
CS32 scFv	4.5
CS39 scFv	~60
27C1/10A6 IgG	9
VT37 scFv	~100

Anti-TGF-β2 antibody	IC_{50}, nM
6A5 scFv	1.5
6A5 IgG	~6
6B1 scFv	0.3
6B1 IgG	0.6
6H1 scFv	0.22
6H1 IgG	~10
11E6 IgG	1.6
14F12 scFv	3
VT37 scFv	2

Table 6 Potency of neutralisation of TGFbeta isoforms

TF1 cell proliferation assay IC_{50} (nM IgG)		
	6B1 IgG4	Genzyme
TGFbeta1	>100	1.5
TGFbeta2	2	10
TGFbeta3	11	0.1
A549 cell radioreceptor assay IC_{50} (nM IgG)		
	6B1 IgG4	Genzyme
TGFbeta1	>400	0.55
TGFbeta2	0.05	0.5
TGFbeta3	4	0.03

Figure 1 Sequences of antibodies specific for TGFbeta1

(a) Antibodies to TGFbeta1 isolated directly from repertoires

(i) 1B2 VH (also known as 7A3 VH)

Sequence Range: 1 to 369

```
              10                 20                 30                 40
       *       *       *         *       *       *       *       *       *
CAG GTG CAA CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

50                60                 70                 80                 90
*         *       *         *        *       *       *       *       *       *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

   100               110                120                130                140
     *       *       *        *         *       *       *       *       *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

       150               160                170                180                190
*       *       *        *       *       *       *       *       *       *
GCA GTT ATA TCA TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

           200               210                220                230                240
   *       *       *       *       *       *       *       *       *       *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>
```

```
           250         260         270         280
     *      *     *     *     *     *     *     *     *
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

290         300         310         320         330
 *     *     *     *     *     *     *     *     *     *
GCG AAA ACT GGG GAA TAT AGT GGC TAC GAT TCT AGT GGT GTG GAC GTC
 A   K   T   G   E   Y   S   G   Y   D   S   S   G   V   D   V>
___a___a___a____TRANSLATION OF 7A3 VH.SEQ [A]__a___a___a___a___>

   340         350         360
     *     *     *     *     *     *
TGG GGC AAA GGG ACC ACG GTC ACC GTC TCC TCA
 W   G   K   G   T   T   V   T   V   S   S>
___a__TRANSLATION OF 7A3 VH.SEQ [A]____a___>
```

(ii) 1A-E5 VH

Sequence Range: 1 to 345

```
            10          20          30          40
     *      *     *     *     *     *     *     *     *
GAG GTG CAG CTG GTG GAG TCT GGT GGA GGC TTA GTT CAG CCT GGG GGG
 E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAC
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

   100         110         120         130         140
     *     *     *     *     *     *     *     *     *
```

3

```
TGG ATG CAC TGG GTC CGC CAA GCT CCA GGG AAG GGG CTG GTG TGG GTC
 W   M   H   W   V   R   Q   A   P   G   K   G   L   V   W   V>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

        150             160             170             180             190
 *       *       *       *       *       *       *       *       *       *
TCA CGT ATT AAT AGT GAT GGG AGT AGC ACA AGC TAC GCG GAC TCC GTG
 S   R   I   N   S   D   G   S   S   T   S   Y   A   D   S   V>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

            200             210             220             230             240
   *       *       *       *       *       *       *       *       *       *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   A   K   N   T   L   Y>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

                250             260             270             280
       *       *       *       *       *       *       *       *       *
CTG CAA ATG AAC AGT CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

290             300             310             320             330
 *       *       *       *       *       *       *       *       *       *
GCA AGG GAG AAT AGT TAT GTG CCT TGG GGG CAG GGC ACC CTG GTC ACC
 A   R   E   N   S   Y   V   P   W   G   Q   G   T   L   V   T>
___a___a___a___a_TRANSLATION OF 1AE-5 VH [A]___a___a___a___a___>

    340
     *       *
GTC TCC TCA
 V   S   S>
___a___a___>
```

(iii) 1A-H6 VH

4

Sequence Range: 1 to 354

```
              10          20          30          40
        *     *     *     *     *     *     *     *     *
CAG GTG CAA CTG CAG GAG TCG GGG GGA GGC GTG GTC CAG CCT GGG GGG
 Q   V   Q   L   Q   E   S   G   G   G   V   V   Q   P   G   G>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT GGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   G   Y>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

   100         110         120         130         140
    *     *     *     *     *     *     *     *     *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

      150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GCA TCT GTA CGG AAC GAT GGA AGT AAT ACA TAC TAC ACA GAC TCC GTG
 A   S   V   R   N   D   G   S   N   T   Y   Y   T   D   S   V>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

         200         210         220         230         240
    *     *     *     *     *     *     *     *     *     *
AAG GGC CGA TTC ACC ATC CCC AGA GAC AAC ACC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   P   R   D   N   T   K   N   T   L   Y>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

            250         260         270         280
       *     *     *     *     *     *     *     *     *
CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCC GTA TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
```

```
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

290         300          310         320         330
 *      *    *     *      *     *     *     *     *     *
ACG TCT GAT CCT TTA CGC TAT CCT ATT GAC TAC TGG GGC CAG GGA ACC
 T   S   D   P   L   R   Y   P   I   D   Y   W   G   Q   G   T>
___a___a___a___a_TRANSLATION OF 1AH-6 VH [A]___a___a___a___a___>

    340         350
     *     *     *
CTG GTC ACC GTC TCG AGT
 L   V   T   V   S   S>
___TRANSLATION OF 1____>
```

(iv) 31G9 VH

Sequence Range: 1 to 369

```
            10          20          30          40
     *       *     *     *     *     *     *     *     *
CAG GTG CAG CTG GTG CAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   Q   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

50          60          70          80          90
 *      *    *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

    100         110         120         130         140
     *     *     *     *     *     *     *     *     *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>
```

```
     150         160         170         180         190
*     *     *     *     *     *     *     *     *     *
GCA GTT ATA TCA TAT GAT GGA AGT ATT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   I   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

       200         210         220         230         240
  *     *     *     *     *     *     *     *     *     *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

           250         260         270         280
     *     *     *     *     *     *     *     *     *
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

290         300         310         320         330
 *     *     *     *     *     *     *     *     *     *
GCG CGA ACT GGT GAA TAT AGT GGC TAC GAT ACG AGT GGT GTG GAG CTC
 A   R   T   G   E   Y   S   G   Y   D   T   S   G   V   E   L>
___a___a___a____TRANSLATION OF 31G9 VH.SEQ [A]_a___a___a___a___>

    340         350         360
     *     *     *     *     *     *
TGG GGG CAA GGG ACC ACG GTC ACC GTC TCC TCA
 W   G   Q   G   T   T   V   T   V   S   S>
___a__TRANSLATION OF 31G9 VH.SEQ [A]
```

(v) 31G9 VL

```
           10          20          30          40
     *     *     *     *     *     *     *     *     *
GAC ATC GTG ATG ACC CAG TCT CCT TCC ACC CTG TCT GCA TCT GTA GGA
```

```
 D   I   V   M   T   Q   S   P   S   T   L   S   A   S   V   G>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

50         60          70          80          90
 *     *     *     *     *     *     *     *     *     *
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGT ATT AGT AGC TGG
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   S   W>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

  100        110         120         130         140
   *     *     *     *     *     *     *     *     *
TTG GCC TGG TAT CAG CAG AAA CCA GGG AGA GCC CCT AAG GTC TTG ATC
 L   A   W   Y   Q   Q   K   P   G   R   A   P   K   V   L   I>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

    150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

       200         210         220         230         240
  *     *     *     *     *     *     *     *     *     *
AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   D   F   T   L   T   I   S   S   L   Q   P>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

           250         260         270         280
     *     *     *     *     *     *     *     *     *
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCG TGG
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   W>
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_a___a___a___a___>

290         300         310         320
 *     *     *     *     *     *     *
ACG TTC GGC CAA GGG ACC AAG CTG GAG ATC AAA CGT
 T   F   G   Q   G   T   K   L   E   I   K   R
```

```
___a___a___a____TRANSLATION OF 31G9 VL.SEQ [A]_
```

Figure 1 (b) Light chains of antibodies to TGFbeta1 isolated by chain shuffling

(i) 7A3 VL

Sequence Range: 1 to 342

```
             10         20          30          40
      *      *     *     *     *    *     *     *     *
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT GTG TCT CTG GGC
 D   I   V   M   T   Q   S   P   D   S   L   A   V   S   L   G>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

50             60         70          80          90
 *      *      *     *     *    *     *     *     *     *
GAG AGG GCC ACC ATC AAC TGC AAG TCC AGC CAG AGT CTT TTA TAC AGC
 E   R   A   T   I   N   C   K   S   S   Q   S   L   L   Y   S>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

  100          110         120         130         140
    *      *     *     *     *    *     *     *     *
TAC AAC AAG ATG AAC TAC TTA GCT TGG TAC CAG CAG AAA CCA GGA CAG
 Y   N   K   M   N   Y   L   A   W   Y   Q   Q   K   P   G   Q>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

     150         160         170         180          190
*      *     *     *     *    *     *     *     *     *
CCT CCT AAG CTG CTC ATT AAC TGG GCA TCT ACC CGG GAA TCC GGG GTC
 P   P   K   L   L   I   N   W   A   S   T   R   E   S   G   V>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

         200         210         220         230         240
  *      *     *     *     *    *     *     *     *     *
CCT GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC
 P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   T>
```

```
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

          250          260          270          280
     *     *     *     *     *     *     *     *     *
ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA GTT TAT TAC TGT CAG CAA
 I   S   S   L   Q   A   E   D   V   A   V   Y   Y   C   Q   Q>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

290          300          310          320          330
 *     *     *     *     *     *     *     *     *     *
TAT TAT GCA ACT CCT CTG ACG TTC GGC CAC GGG ACC AAG GTG GAA ATC
 Y   Y   A   T   P   L   T   F   G   H   G   T   K   V   E   I>
___a___a___a____TRANSLATION OF 7A3 VL.SEQ [A]__a___a___a___a___>

  340
   *
AAA CGT
 K   R>
___a___>
```

(ii) <u>10A6 VL</u>

Sequence Range: 1 to 357

```
           10          20          30          40
     *     *     *     *     *     *     *     *     *
CAC GTT ATA CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 H   V   I   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
ACA GTC AGG ATC ACG TGC CAA GGA GAC AGC CTC AAA AGC TAC TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   K   S   Y   Y   A>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

   100         110         120         130         140
```

```
      *   *    *    *    *    *    *    *    *
AGT TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

     150         160         170         180         190
 *    *    *    *    *    *    *    *    *    *    *
GGT GAA AAC AGC CGG CCC TCC GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   E   N   S   R   P   S   G   I   P   D   R   F   S   G   S>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

        200         210         220         230         240
  *      *    *    *    *    *    *    *    *    *
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

           250         260         270         280
      *    *    *    *    *    *    *    *    *
GAT GAA GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT GGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   G   T   H>
___a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>

290         300         310         320         330
 *    *    *    *    *    *    *    *    *
CTA GAA GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 L   E   V   F   G   G   G   T   K   L   T   V   L   G
    a___a___a____TRANSLATION OF 10A6 VL.SEQ [A]_a___a___a___a___>
```

Figure 1 (c) Antibodies to TGFbeta1 isolated from CDR3 spiking experiment

(i) 27C1 VH

Sequence Range: 1 to 369

```
            10          20          30          40
```

```
        *         *         *         *         *         *         *         *         *
CAG GTG CAA CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

50              60               70              80              90
 *       *       *        *        *       *       *       *       *       *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA CTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   L   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

  100             110              120              130              140
   *       *       *        *        *        *        *       *       *
GAC ATG CAC TGG GTC CGC CAG CCT CCA GCC AAG GGG CTG GAG TGG GTG
 D   M   H   W   V   R   Q   P   P   A   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

     150              160              170              180              190
*      *       *        *       *       *       *       *       *       *
GCA GTT ATA TCA TAT GAT GGA AGT AGT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   S   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

        200              210              220              230              240
  *      *       *       *       *       *       *       *       *       *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

          250              260              270              280
      *       *       *       *       *       *       *       *       *
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

290              300              310              320              330
 *       *       *       *       *       *       *       *       *       *
```

```
GCG CGA ACT GGT GAA TAT AGT GGC TAC GAC ACG AGT GGT GTG GAG CTC
 A   R   T   G   E   Y   S   G   Y   D   T   S   G   V   E   L>
___a___a___a____TRANSLATION OF 27C1 VH.SEQ [A]_a___a___a___a___>

 340          350          360
   *     *     *     *     *     *
TGG GGG CAA GGG ACC ACG GTC ACC GTC TCC TCA
 W   G   Q   G   T   T   V   T   V   S   S>
___a__TRANSLATION OF 27C1 VH.SEQ [A]___a___>
```

Figure 2 Sequences of antibodies specific for TGFbeta2

(a) Antibodies to TGFbeta 2 isolated directly from repertoires

(i) 2A-H11 VH (also known as 6H1 VH)

Sequence Range: 1 to 345

```
                10          20          30          40
        *       *     *     *     *     *     *     *     *
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

  100         110         120         130         140
   *     *     *     *     *     *     *     *     *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

    150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

      200         210         220         230         240
   *     *     *     *     *     *     *     *     *     *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>
```

```
                    250                 260                 270                 280
         *         *        *         *         *        *         *         *        *
CTG CAA ATG GAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   D   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

290                 300                 310                 320                 330
 *         *        *         *         *        *         *         *        *         *
GGA AGA ACG CTG GAG TCT AGT TTG TGG GGC CAA GGC ACC CTG GTC ACC
 G   R   T   L   E   S   S   L   W   G   Q   G   T   L   V   T>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

    340
     *       *
GTC TCC TCA
 V   S   S>
___a___a___>
```

(ii) <u>2A-A9 (also known as 11E6 VH)</u>

Sequence Range: 1 to 350

```
                     10                  20                  30                  40
         *         *        *         *         *        *         *         *        *
GAG ATT CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGA
 E   I   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

50                  60                  70                  80                  90
 *         *        *         *         *        *         *         *        *         *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

    100                 110                 120                 130                 140
     *        *         *         *        *         *         *        *         *
```

```
GCT ATG CAC TGG GTC CGC CAG GCT CCA GCC AAG GGG CTG GAG TGG GTG
 A   M   H   W   V   R   Q   A   P   A   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

        150             160             170             180             190
 *       *       *       *       *       *       *       *       *       *
GCA GTT ATA TCA TAT GAT GGA AGC AAT AAA TAC TAC GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

            200             210             220             230             240
   *         *       *       *       *       *       *       *       *       *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

                250             260             270             280
       *         *       *       *       *       *       *       *       *
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

290             300             310             320             330
 *       *       *       *       *       *       *       *       *       *
GCA AGA GCG GGG TTG GAA ACG ACG TGG GGC CAA GGA ACC CTG GTC ACC
 A   R   A   G   L   E   T   T   W   G   Q   G   T   L   V   T>
___a___a___a____TRANSLATION OF 11E6 VH.SEQ [A]_a___a___a___a___>

    340             350
     *       *       *
GTC TCC TCA AGT GG
 V   S   S   S   G>
___TRANSLATION_a__>
```

(iii) <u>Gold11-VH</u>

Sequence Range: 1 to 369

```
                10         20         30         40
         *      *    *     *     *    *     *     *    *
CAG GTC ACC TTG AAG GAG TCT GGG GGA AGC GTG GTC CAG CCT GGG AGG
 Q   V   T   L   K   E   S   G   G   S   V   V   Q   P   G   R>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

50           60          70          80          90
 *     *     *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

  100         110         120         130         140
     *     *     *     *     *     *     *     *     *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

    150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GCA GTT ATA TCA TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

       200         210         220         230         240
   *     *     *     *     *     *     *     *     *     *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CAG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   Q   Y>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

          250         260         270         280
```

```
                          *         *         *         *         *         *         *         *         *
CTG CAA ATG AAC AGC CTG AGA GCT GAA GAC ACG GCA GAG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   E   Y   Y   C>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

290                300                310               320               330
 *        *         *         *         *         *         *         *         *         *
GCG AGA ACT GGG GAA TAT AGT GGC CAC GCA TCT ACT GGA GAG AAC GTC
 A   R   T   G   E   Y   S   G   H   A   S   T   G   E   N   V>
___a___a___a___a_TRANSLATION OF GOLD11-VH [A]__a___a___a___a___>

     340                350                360
      *         *         *         *         *         *
TGG GGC CGG GGC ACC CTG GTC ACC GTC TCG AGT
 W   G   R   G   T   L   V   T   V   S   S>
___a___TRANSLATION OF GOLD11-VH [A]_____a___>
```

(iv) Gold11-VL

Sequence Range: 1 to 336

```
                     10                  20                  30                  40
          *         *         *         *         *         *         *         *         *
TCC TAT GTG CTG ACT CAC CCC CCC TCA GTG TCT GGG ACC CCC GGG CAG
 S   Y   V   L   T   H   P   P   S   V   S   G   T   P   G   Q>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

50                   60                  70                  80                  90
 *        *         *         *         *         *         *         *         *         *
AGA GTC ACC ATC TCT TGT TCT GGA GGC AGA TCC AAC ATC GGC AGT AAT
 R   V   T   I   S   C   S   G   G   R   S   N   I   G   S   N>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

     100                110                120                130                140
      *         *         *         *         *         *         *         *         *
ACT GTA AAG TGG TAT CAG CAG CTC CCA GGA ACG CCC CCC AAA CTC CTC
 T   V   K   W   Y   Q   Q   L   P   G   T   P   P   K   L   L>
```

```
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

    150         160         170         180         190
*    *     *     *     *     *     *     *     *     *
ATC TAT GGC AAT GAT CAG CGG CCC TCA GGG ATC CCT GAC CGA TTC TCT
 I   Y   G   N   D   Q   R   P   S   G   I   P   D   R   F   S>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

        200         210         220         230         240
 *       *     *     *     *     *     *     *     *     *
GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT GGG GTC CAG
 G   S   K   S   G   T   S   A   S   L   A   I   T   G   V   Q>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

            250         260         270         280
     *       *     *     *     *     *     *     *     *
GCT GAA GAC GAG GCT GAC TAT TAC TGC CAG TCA TAT GAC AGC AGC CTG
 A   E   D   E   A   D   Y   Y   C   Q   S   Y   D   S   S   L>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>

290         300         310         320         330
 *     *     *     *     *     *     *     *     *     *
AGG GGT TCG AGG GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA GGT
 R   G   S   R   V   F   G   T   G   T   K   V   T   V   L   G>
___a___a___a___a_TRANSLATION OF GOLD11-VL [A]__a___a___a___a___>
```

(v) <u>1-G2</u>

Sequence Range: 1 to 381

```
            10          20          30          40
     *       *     *     *     *     *     *     *     *
CAG GTA CAA CCT CAG CAG TCT GGG GGA GAG GTG AAG CAG CCT GGG GCC
 Q   V   Q   P   Q   Q   S   G   G   E   V   K   Q   P   G   A>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

50          60          70          80          90
```

```
 *     *     *     *     *     *     *     *     *     *
TCC GTG AAG GTT TCC TGT AAG GCG TCT GGA TAC ACC TTC ACC AGC TTC
 S   V   K   V   S   C   K   A   S   G   Y   T   F   T   S   F>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

    100         110         120         130         140
     *     *     *     *     *     *     *     *     *
TAT ATG AAC TGG GTG CGA CAG GCC CCC GGA CAA GGG CTT GAG TGG ATG
 Y   M   N   W   V   R   Q   A   P   G   Q   G   L   E   W   M>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

       150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GGA ATA ATC AGC CCT CGT GGT GGT ACG ACA AGT TAC GCA CAG AAC TTC
 G   I   I   S   P   R   G   G   T   T   S   Y   A   Q   N   F>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

           200         210         220         230         240
   *     *     *     *     *     *     *     *     *     *
CAG GGC AGA GTC ACC ATG ACC AGG GAC ACG TCC ACA AGC ACA GTC TAC
 Q   G   R   V   T   M   T   R   D   T   S   T   S   T   V   Y>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

              250         260         270         280
       *     *     *     *     *     *     *     *     *
ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC GTG TAT TAT TGT
 M   E   L   S   S   L   R   S   E   D   T   A   V   Y   Y   C>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

290               300         310         320         330
 *     *     *     *     *     *     *     *     *     *
GCG ATA ATT GGG GGT ACT ACT ATG AGA GTA GGG GGG CCC GAT GCT TTT
 A   I   I   G   G   T   T   M   R   V   G   G   P   D   A   F>
___a___a___a___a__TRANSLATION OF 1-G2-VH [A]___a___a___a___a___>

    340         350         360         370         380
     *     *     *     *     *     *     *     *     *
```

```
GAT ATC TGG GGC CAA GGG ACA ATG GTC ACC GTC ACC GTC TCT TCA
 D   I   W   G   Q   G   T   M   V   T   V   T   V   S   S>
___a___a___a____TRANSLATION OF 1-G2-VH [A]_a___a___a___a___>
```

(vi) 1-H6

Sequence Range: 1 to 381

```
             10          20          30          40
     *        *     *     *     *     *     *     *     *
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGG AAC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   R   N   Y>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

  100        110         120         130         140
     *     *     *     *     *     *     *     *     *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

     150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

        200         210         220         230         240
  *      *     *     *     *     *     *     *     *     *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
```

```
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

          250         260         270         280
     *     *     *     *     *     *     *     *     *
CTG CAA ATG AAC AGC CTG AGA GTC GAG GAC ACG GCT GTT TAT TAC TGT
 L   Q   M   N   S   L   R   V   E   D   T   A   V   Y   Y   C>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

290         300         310         320         330
 *     *     *     *     *     *     *     *     *     *
GCG AGA AGA TGG TAT GGT GGC AGT GGT TAT TGG GGC CAC TTC TAC TCC
 A   R   R   W   Y   G   G   S   G   Y   W   G   H   F   Y   S>
___a___a___a___a__TRANSLATION OF 1-H6 VH [A]___a___a___a___a___>

   340         350         360         370         380
     *     *     *     *     *     *     *     *     *
TAC ATG GAC GGC TGG GGC AAA GGG ACC AAG GTC ACC GTC TCC TCA
 Y   M   D   G   W   G   K   G   T   K   V   T   V   S   S>
___a___a___a____TRANSLATION OF 1-H6 VH [A]_a___a___a___a___>
```

Figure 2(b) Light chains of antibodies specific for TGFbeta2 isolated following chain shuffling

(i) 6H1 VL

Sequence Range: 1 to 348

```
            10          20          30          40
     *     *     *     *     *     *     *     *     *
GAT GTT GTG ATG ACT CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA
 D   V   V   M   T   Q   S   P   S   S   L   S   A   S   V   G>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>
```

```
50        60         70         80         90
 *    *    *    *    *    *    *    *    *    *
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGC ATT AGC AAT TAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

   100        110        120        130        140
    *    *    *    *    *    *    *    *    *
TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

      150        160        170        180        190
 *    *    *    *    *    *    *    *    *    *
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGT GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

         200        210        220        230        240
   *    *    *    *    *    *    *    *    *    *
AGT GGA TCT GGG ACA GAA TTC ACT CTC ACA ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   E   F   T   L   T   I   S   S   L   Q   P>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

           250        260        270        280
       *    *    *    *    *    *    *    *    *
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT CGA
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   R>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

290        300        310        320        330
 *    *    *    *    *    *    *    *    *    *
ACG TTC GGC CAA GGG ACC AAA GTG GAT ATC AAA CGT
 T   F   G   Q   G   T   K   V   D   I   K   R
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>
```

(ii) 6A5 VL

Sequence Range: 1 to 327

```
              10          20          30          40
       *      *      *      *      *      *      *      *      *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

50          60          70          80          90
 *      *      *      *      *      *      *      *      *
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

  100         110         120         130         140
   *      *      *      *      *      *      *      *      *
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

     150         160         170         180         190
*      *      *      *      *      *      *      *      *      *
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC GCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   A   G   S>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

        200         210         220         230         240
  *      *      *      *      *      *      *      *      *      *
AAC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAG
 N   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

          250         260         270         280
       *      *      *      *      *      *      *      *      *
GAT GAG GCT GAC TAT TAC TGT AGC TCC CGG GAC AGC AGT GGT AAC CAT
```

```
 D   E   A   D   Y   Y   C   S   S   R   D   S   S   G   N   H>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

290            300             310             320
 *       *      *       *       *       *       *       *
GTG GTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   V   F   G   G   G   T   K   L   T   V   L   G>
___a___a__TRANSLATION OF 6A5 VL.SEQ [A]____a___a___>
```

(iii) 6B1 VL

Sequence Range: 1 to 330

```
               10              20              30              40
     *       *       *       *       *       *       *       *       *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

50              60              70              80              90
 *       *       *       *       *       *       *       *       *       *
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

   100             110             120             130             140
     *       *       *       *       *       *       *       *       *
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

       150             160             170             180             190
 *       *       *       *       *       *       *       *       *       *
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>
```

```
            200            210              220             230             240
  *          *       *      *       *        *       *       *       *       *
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

               250            260             270            280
      *         *      *       *       *       *       *       *       *
GAT GAG GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT AGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   S   T   H>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

290            300             310            320            330
 *       *      *       *       *       *       *       *      *
CGA GGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 R   G   V   F   G   G   G   T   K   L   T   V   L   G>
___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___>
```

(IV) <u>11E6 VL</u>

Sequence Range: 1 to 324

```
                 10             20              30              40
      *          *       *      *       *       *       *       *       *
GAA GTT GTG CTG ACT CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA
 E   V   V   L   T   Q   S   P   S   S   L   S   A   S   V   G>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

50             60              70             80              90
 *       *      *       *       *       *       *       *       *       *
GAC AGA GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT GGA GAT GAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   G   D   D>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

    100             110             120              130             140
```

```
      *       *       *       *       *       *       *       *       *
TTG GGC TGG TAT CAG CAG AAG CCA GGG AAA GCC CCT ATC CTC CTG ATC
 L   G   W   Y   Q   Q   K   P   G   K   A   P   I   L   L   I>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

       150          160          170          180          190
 *      *       *       *       *       *       *       *       *
TAT GGT ACA TCC ACT TTA CAA AGT GGG GTC CCG TCA AGG TTC AGC GGC
 Y   G   T   S   T   L   Q   S   G   V   P   S   R   F   S   G>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

           200          210          220          230          240
  *         *       *       *       *       *       *       *       *       *
AGT GGA TCT GGC ACA GAT TTC ACT CTC ACC ATC AAC AGC CTG CAG CCT
 S   G   S   G   T   D   F   T   L   T   I   N   S   L   Q   P>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

               250          260          270          280
      *       *       *       *       *       *       *       *       *
GAA GAT TTT GCA ACT TAT TAC TGT CTA CAA GAT TCC AAT TAC CCG CTC
 E   D   F   A   T   Y   Y   C   L   Q   D   S   N   Y   P   L>
___a___a___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___a___a___>

290             300          310          320
 *       *       *       *       *       *       *
ACT TTC GGC GGA GGG ACA CGA CTG GAG ATT AAA CGT
 T   F   G   G   G   T   R   L   E   I   K   R>
___a____TRANSLATION OF 11E6 VL.SEQ [A]_a___a___>
```

(v) <u>14F12 VL</u>

Sequence Range: 1 to 321

```
               10           20           30           40
      *       *       *       *       *       *       *       *       *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
```

```
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

50             60                70              80              90
 *       *       *       *       *       *       *       *       *       *
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AAC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   N   Y   Y   A>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

  100            110             120             130             140
   *       *       *       *       *       *       *       *       *
AAC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 N   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

    150             160             170             180             190
*       *       *       *       *       *       *       *       *       *
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

        200             210             220             230             240
  *       *       *       *       *       *       *       *       *       *
AGC TCA GGG AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CGG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   R   A   E>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

           250             260             270             280
    *       *       *       *       *       *       *       *       *
GAT GAG GGT GTC TAT TAC TGT AAC TCC CGG GAC AGC AGT GGT GCG GTT
 D   E   G   V   Y   Y   C   N   S   R   D   S   S   G   A   V>
___a___a___a___TRANSLATION OF 14F12 VL.SEQ [A]_a___a___a___a___>

290             300             310             320
 *       *       *       *       *       *       *
TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 F   G   G   G   T   K   L   T   V   L   G>
```

___a_TRANSLATION OF 14F12 VL.SEQ [A]____a____>

Figure 3 Sequence of VH CDR3 of spiked clones derived from 1B2

PARENT (1-B2)	A R T G E Y S G Y D S S G V D V W
27-C1	A R T G E Y S G Y D **T** S G V **E** **L** W
27-D7	A R T **R** E Y S G **H** D S S G V D **D** W
27-E10	A R T G **P** **F** S G Y D S S G **E** D V **R**
27-H1	A R T **E** E Y S G Y D S S G V D V W
27-E2	A **Q** T **R** E Y **T** G Y D S S G V D V W
28-A11	A R T **E** E Y S G **F** D S **T** G **E** D V W
28-E12	A R T **E** E **F** S G Y D S S G V D V W
28-H10	A R T G E Y S G Y **H** S S G V D V **R**
31-G2	A R T **E** E **F** S G Y D S S G V D V W
30-B6	A R **A** G **P** **F** S G Y D S S G **E** D V **R**
30-E9	A R T G **P** **F** S G Y D S S G **E** D V W
30-F6	A R T **E** E **F** S G Y D S S G V D V W
30-D2	A R T G E Y S G Y D S S G **E** **L** V W
31-A2	A R T **E** E **F** S G Y D S **T** G E **E** V W
31-E11	A R T **E** E **F** S G Y D S S G V D V W
31-F1	A R T G E Y S G Y D S S G **E** D V W

Differences from 1B2 VH CDR3 are in bold.

Figure 4 Sequence of VT37 VL of antibody cross-reactive between TGFbeta1 and TGFbeta2

Sequence Range: 1 to 327

```
              10          20          30          40
     *        *     *     *     *     *     *     *     *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
ACA GTT AGG ATC ACT TCC CAA GGA GAC AGT CTC AGA AGC TAT TAC ACA
 T   V   R   I   T   S   Q   G   D   S   L   R   S   Y   Y   T>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

   100         110         120         130         140
     *     *     *     *     *     *     *     *     *
AAC TGG TTT CAG CAG AAG CCA GGA CAG CCC CCT CTA CTT GTC GTC TAT
 N   W   F   Q   Q   K   P   G   Q   P   P   L   L   V   V   Y>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

     150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GCT AAA AAT AAG CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 A   K   N   K   R   P   S   G   I   P   D   R   F   S   G   S>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

         200         210         220         230         240
 *     *     *     *     *     *     *     *     *     *
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

           250         260         270         280
     *     *     *     *     *     *     *     *     *
GAT GAG GCT GAC TAT TAC TGT CAT TCC CGG GAC AGC AGT GGT AAC CAT
```

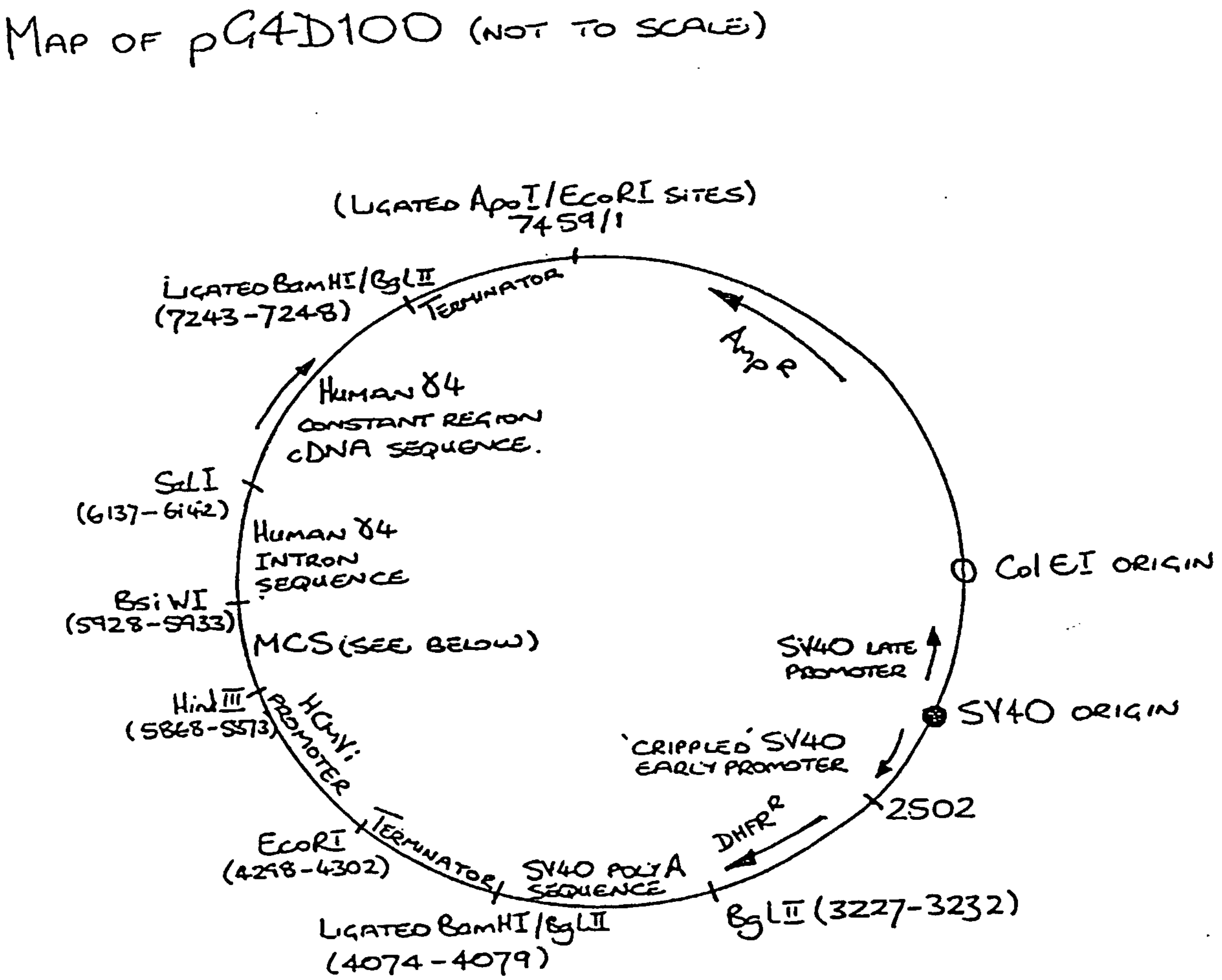
```
 D   E   A   D   Y   Y   C   H   S   R   D   S   S   G   N   H>
___a___a___a___a__TRANSLATION OF VT37-VL [A]___a___a___a___a___>

290            300            310            320
 *       *       *       *       *       *       *       *
GTG CTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   L   F   G   G   G   T   K   L   T   V   L   G>
___a___a____TRANSLATION OF VT37-VL [A]_a___a___a___>
```

Figure 5. vhcassete2

```
DNA sequence of heavy chain VH leader from intermediate vectors

 With 6 enzymes: HINDIII SFII PSTI BSTEII BAMHI ECORI

                              March 30, 1994  09:50  ..

        H
        i
        n
        d
        I
        I
        I
        aagcttgccgccaccatggactggacctggcgcgtgttttgcctgctcgccgtggcccct
      1 ---------+---------+---------+---------+---------+---------+ 60
        ttcgaacggcggtggtacctgacctggaccgcgcacaaaacggacgagcggcaccgggga

a       K  L  A  A  T  M  D  W  T  W  R  V  F  C  L  L  A  V  A  P  -

                                                        B
                                                        s
        S                         P                     t
        f                         s                     E
        i                         t                     I
        I                         I                     I
        ggggcccacagccaggtgcaactgcagcagtccggtgccaagggaccacggtcaccgtct
     61 ---------+---------+---------+---------+---------+---------+ 120
        ccccgggtgtcggtccacgttgacgtcgtcaggccacggttccctggtgccagtggcaga

a       G  A  H  S  Q  V  Q  L  Q  Q  S  G  A  K  G  P  R  S  P  S  -

                    B     E
                    a     c
                    m     o
                    H     R
                    I     I
        cctcaggtgagtggatccgaattc
    121 ---------+---------+---- 144
        ggagtccactcacctaggcttaag

a       P  Q  V  S  G  S  E  F  -

 Enzymes that do cut:

    BamHI    BstEII    EcoRI   HindIII     PstI     SfiI

 Enzymes that do not cut:

   NONE
```

Figure 6 pG4D100

Map



MCS: 5' HindIII – PacI – BamHI – (XcmI) – (PmlI) – (NheI) – AscI – (BssHII) – XhoI – PmeI – BsiWI 3'

NOTE: THOSE R. SITES SHOWN IN BRACKETS ARE NOT UNIQUE.

Figure 7 vlcassetteCAT I

DNA coding for vl leader including intron. ApaLI site in the leader

With 5 enzymes: HINDIII SACI APALI XHOI BAMHI

June 16, 1994 15:44 ..

```
      H
      i
      n
      d
      I
      I
      I
      aagcttcgccaccatgggatggagctgtatcatcctcttcttggtagcaacagctacagg
    1 ---------+---------+---------+---------+---------+---------+ 60
      ttcgaagcggtggtaccctacctcgacatagtaggagaagaaccatcgttgtcgatgtcc

                   M  G  W  S  C  I  I  L  F  L  V  A  T  A  T

      taaggggctcacagtagcaggcttgaggtctggacatatatatgggtgacaatgacatcc
   61 ---------+---------+---------+---------+---------+---------+ 120
      attccccgagtgtcatcgtccgaactccagacctgtatatatacccactgttactgtagg

                             A
                             p                   S
                             a                   a
                             L                   c
                             I                   I
      actttgcctttctctccacaggtgtgcactccgacattgagctcacccagtctccagaca
  121 ---------+---------+---------+---------+---------+---------+ 180
      tgaaacggaaagagaggtgtccacacgtgaggctgtaactcgagtgggtcagaggtctgt

                          G  V  H  S  D  I  E  L

                                                       B
        X                                              a
        h                                              m
        o                                              H
        I                                              I
      aagctcgagctgaaacgtgagtagaatttaaactttgcttcctcaattggatcc
  181 ---------+---------+---------+---------+---------+---- 234
      ttcgagctcgactttgcactcatcttaaatttgaaacgaaggagttaacctagg

        L  E  L  K
```

Enzymes that do cut:

ApaLI BamHI HindIII SacI XhoI

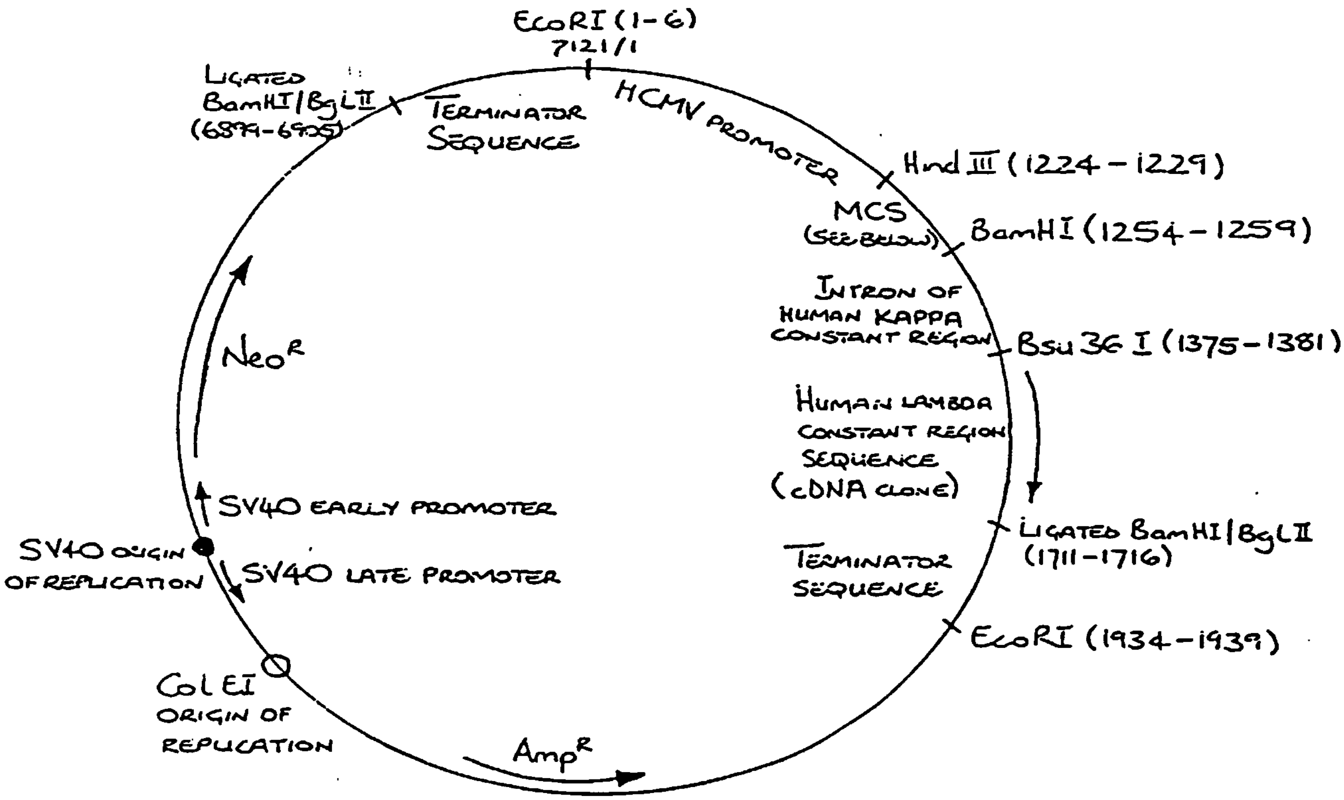
Enzymes that do not cut:

NONE

Figure 8 pLN10

Map

MAP OF pLN10 (NOT TO SCALE)



MULTIPLE CLONING SITE (MCS):

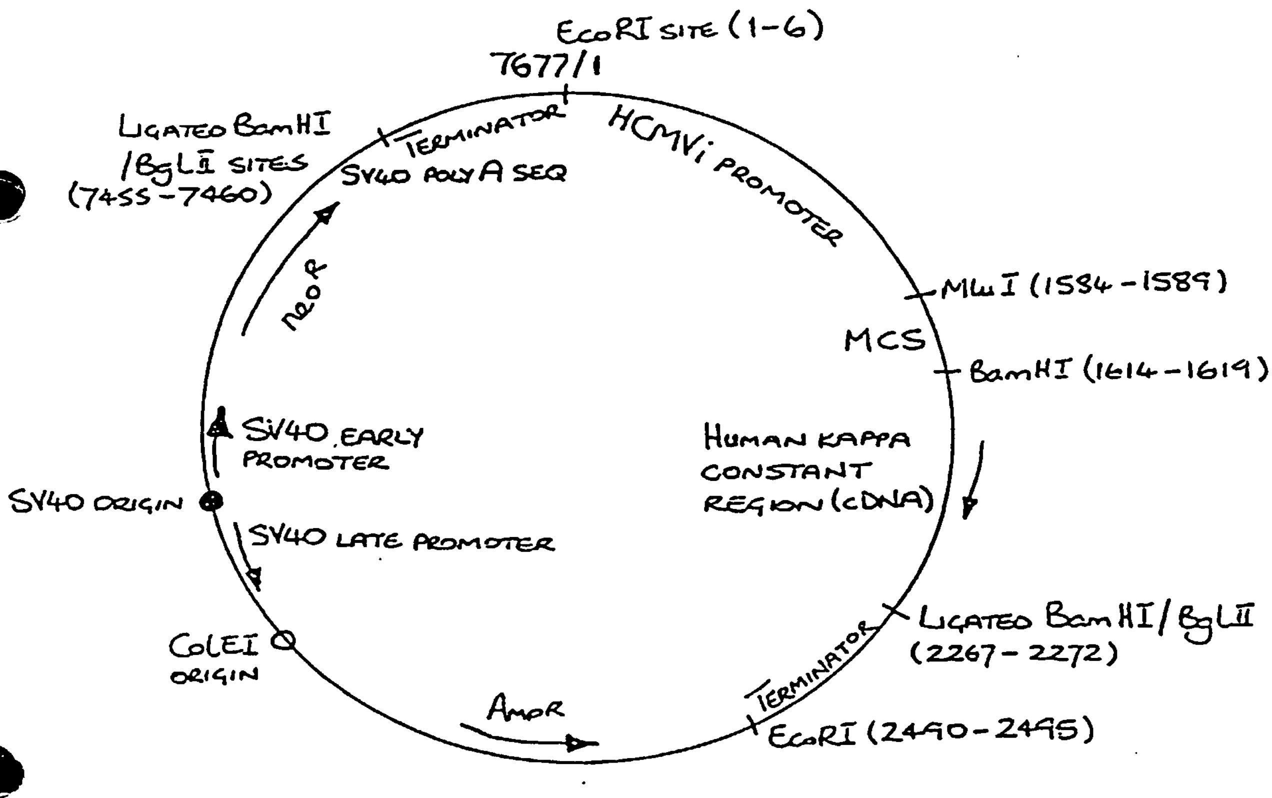
5' Hind III - (SphI) - (PstI) - SalI - XbaI - BamHI 3'
1224 ... 1259

NOTE: RESTRICTION SITES IN BRACKETS ARE NOT UNIQUE.

Figure 9 pKN100

Map

Map of pKN100 (not to scale)



MCS: 5' MluI - (AvaI) - HindIII - (SphI) - (PstI) - SalI - XbaI - BamHI 3'

Fig 10 Neutralisation of TGFβ2 by scFv Antibodies

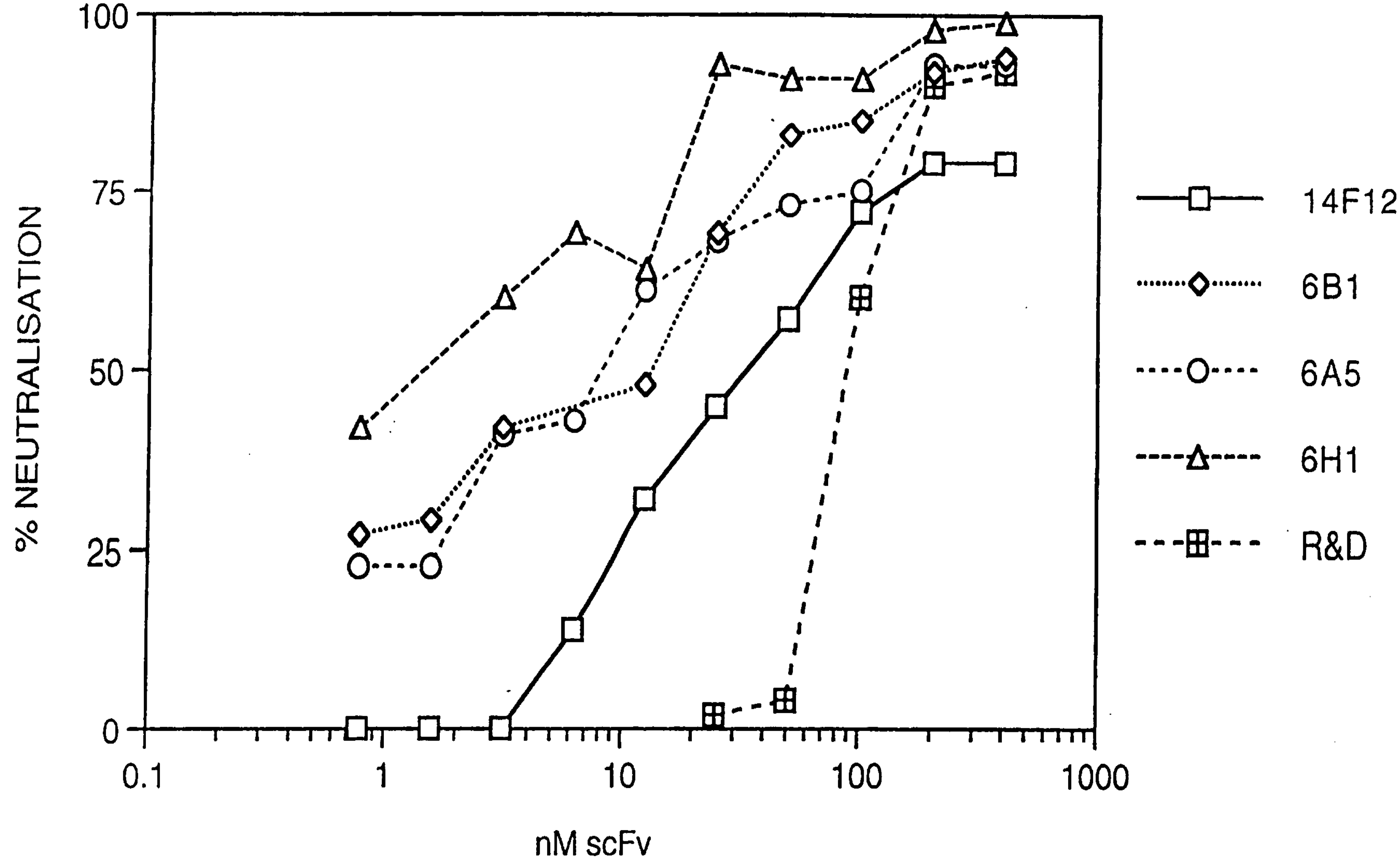


Fig 11 Neutralisation of TGF β_2 by Whole IgG4 Antibodies

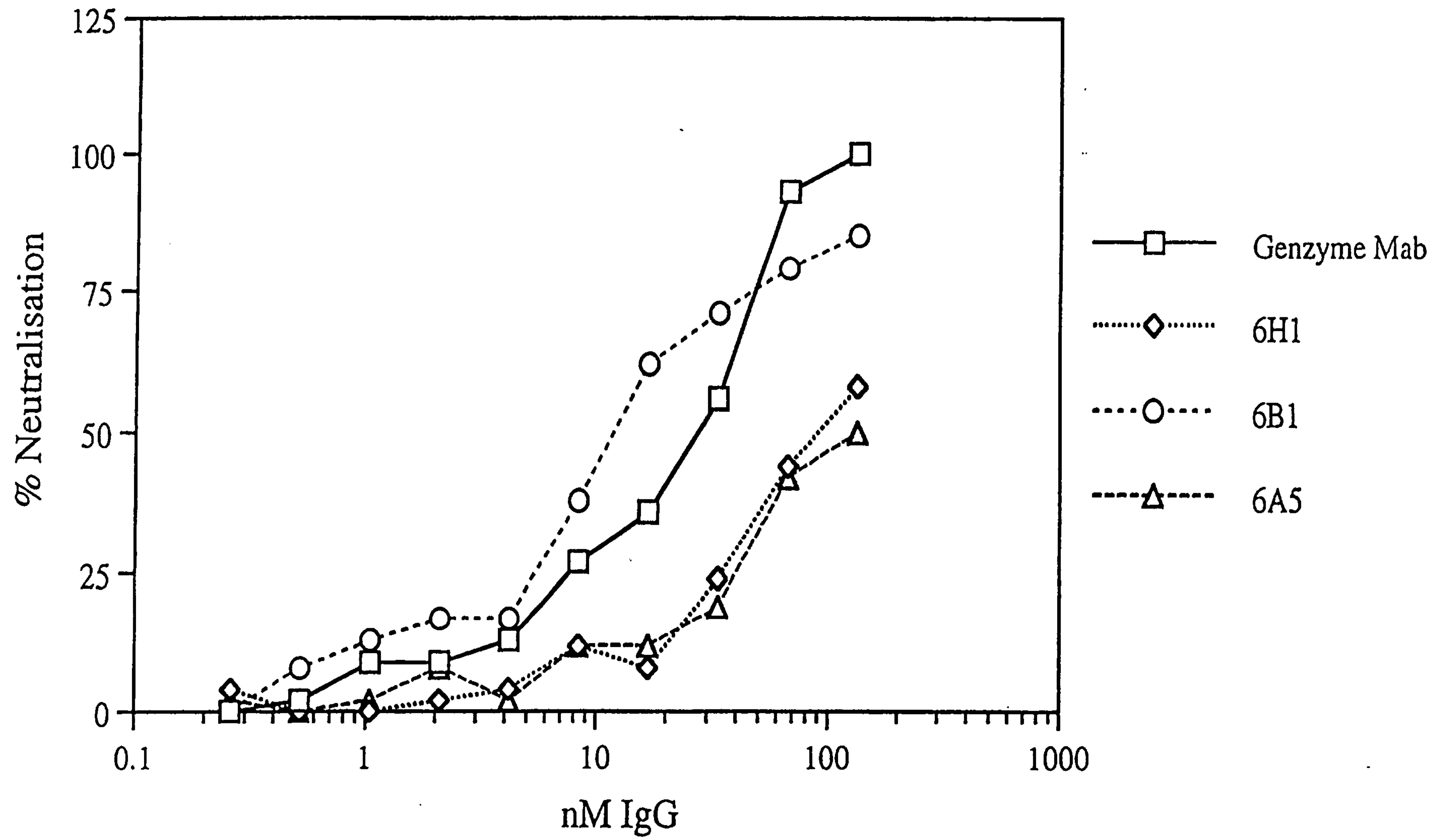
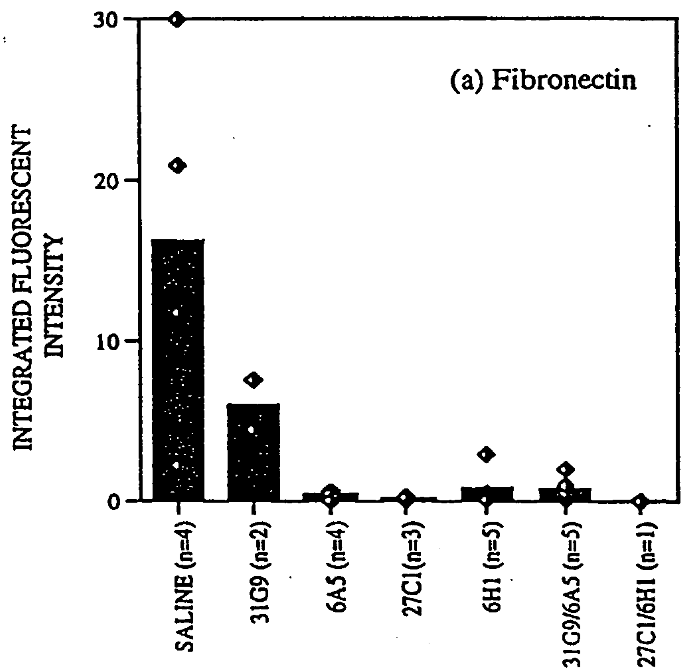


Figure 12.
Scatter plots of individual animal data points. Bar graph is the mean of the group.



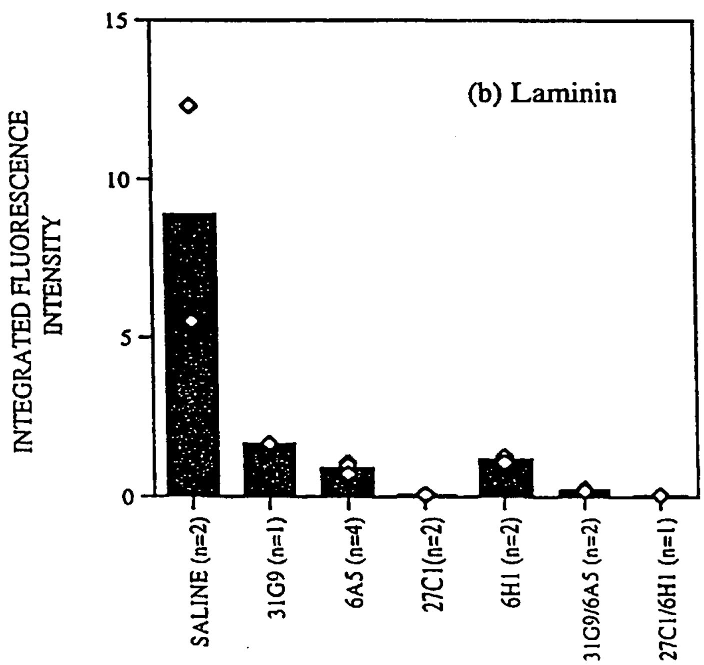
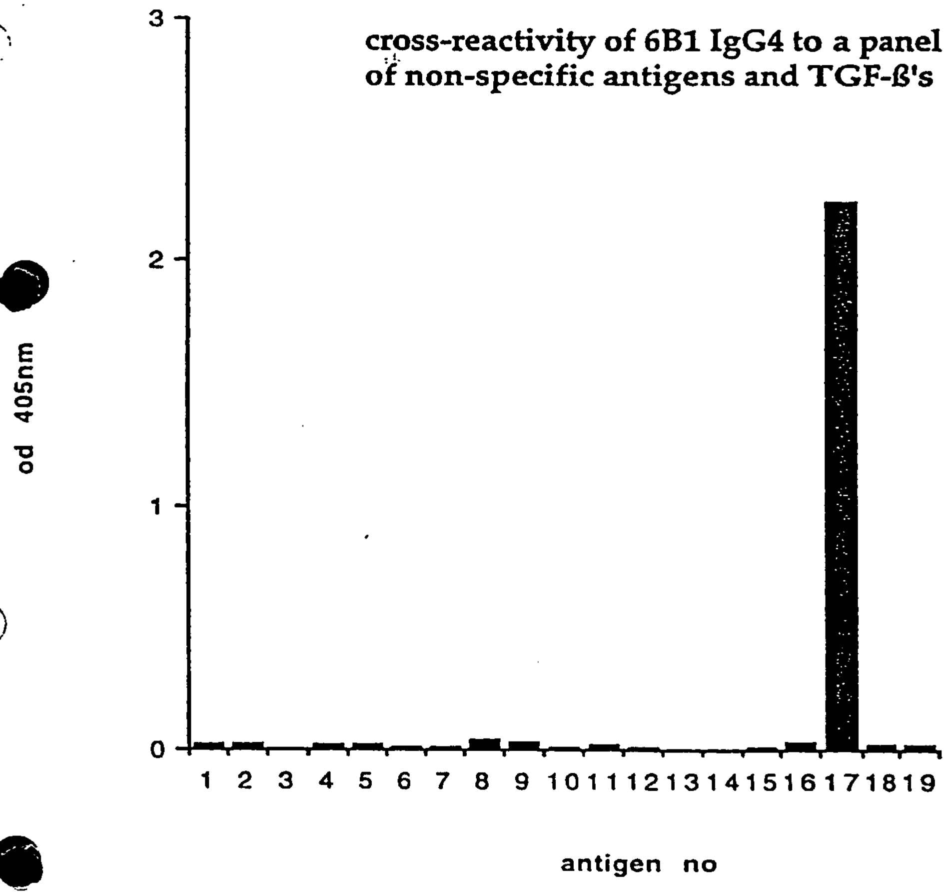
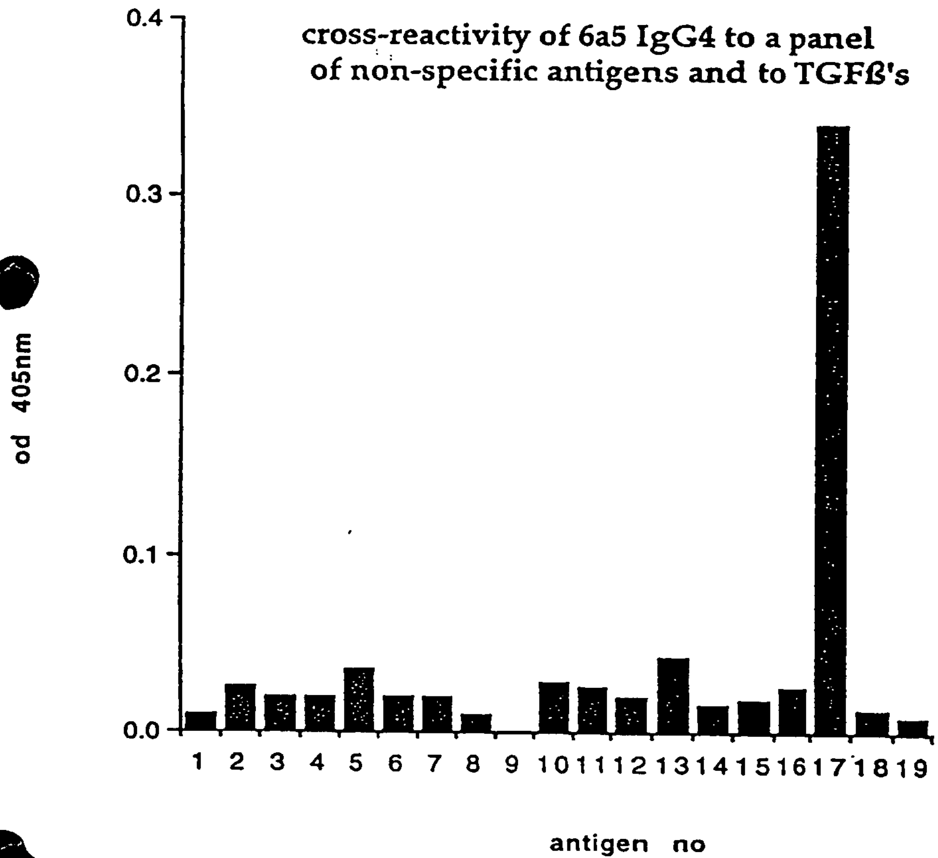


Fig. 13 (a)



1 interleukin 1
2 human lymphotoxin (TNFbeta)
3 human insulin
4 human serum albumin
5 ss DNA
6 oxazolone -bovine serum albumin
7 keyhole limpet haemocyanin
8 chicken egg white trypsin inhibitor
9 chymotrypsinogen
10 cytochrome c
11 GADPH
12 ovalbumin
13 hen egg lysozyme
14 bovine serum albumin
15 TNF-alpha
16 TGF beta 1
17 TGF beta 2
18 TGF beta 3
19 PBS only

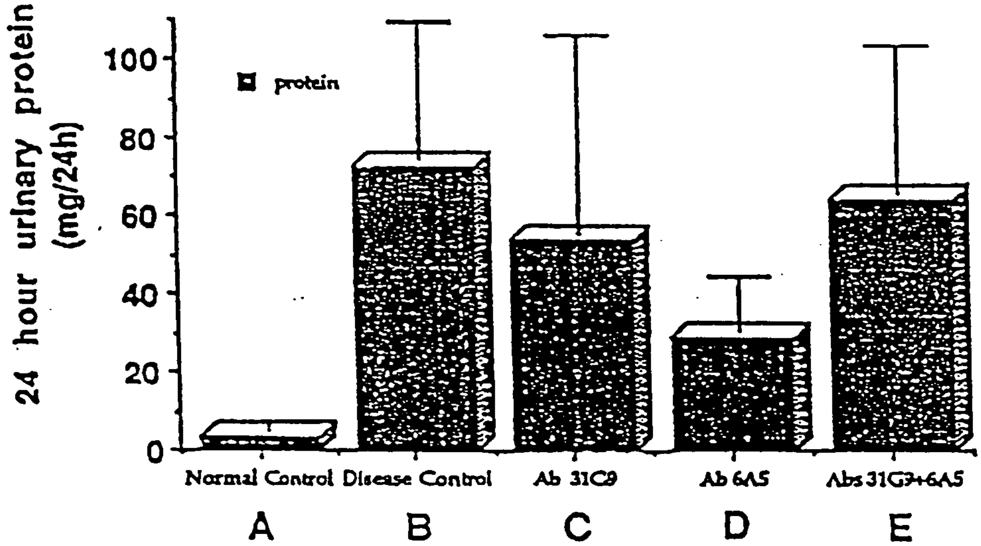
Fig. 13 (b)



For both Fig 13 (a) + (b). Antigens 1 to 15 were used for coating the plate at a concentration of 10 μg/ml in PBS.
The TGFbetas were coated at 0·2 μg/ml in PBS.
Coating was performed at 4°C overnight.

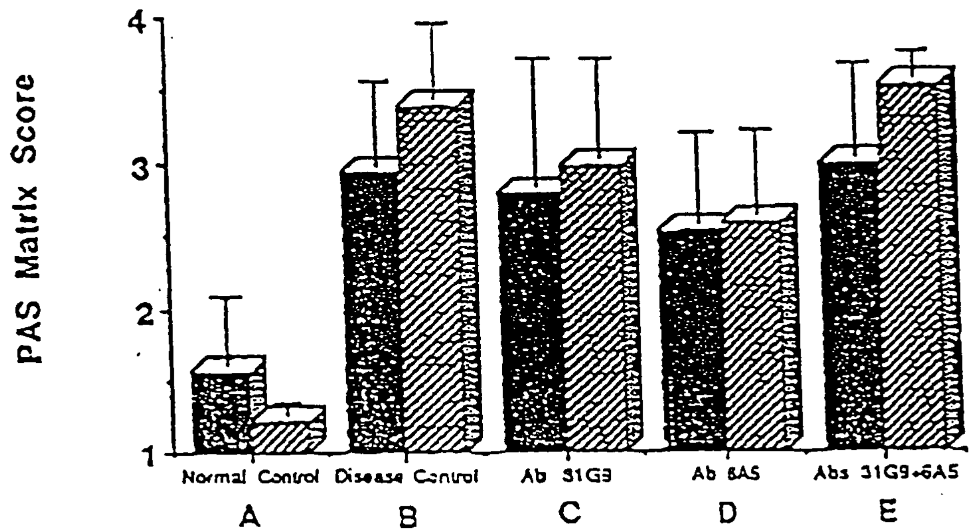
100 μl of each antigen was used per well and duplicates of each antigen for each IgG to be tested. IgG samples were incubated with the coated antigens at 37°C for 2 hours after blocking with 2% marvel-PBS. The labelled second antibody was a mouse anti-human Fc, alkaline phosphatase conjugated and the substrate used to detect bound second antibody was PNPP at 1mg/ml with the absorbance read at 405nm.

FIGURE 14



t-test B/C,B/E,C/D,C/E,D/E =NS; A/B,
A/D =p<0.01; A/E=p<0.02; A/C, B/D =p<0.10

FIGURE 15



t-test stats: B vs A; B vs D:p<0.0001
All other comparisons are NS

NEUTRALISATION OF TGF BETA 2 ANTI-PROLIFERATIVE EFFECT
ON TF1 CELLS BY WHOLE ANTIBODIES

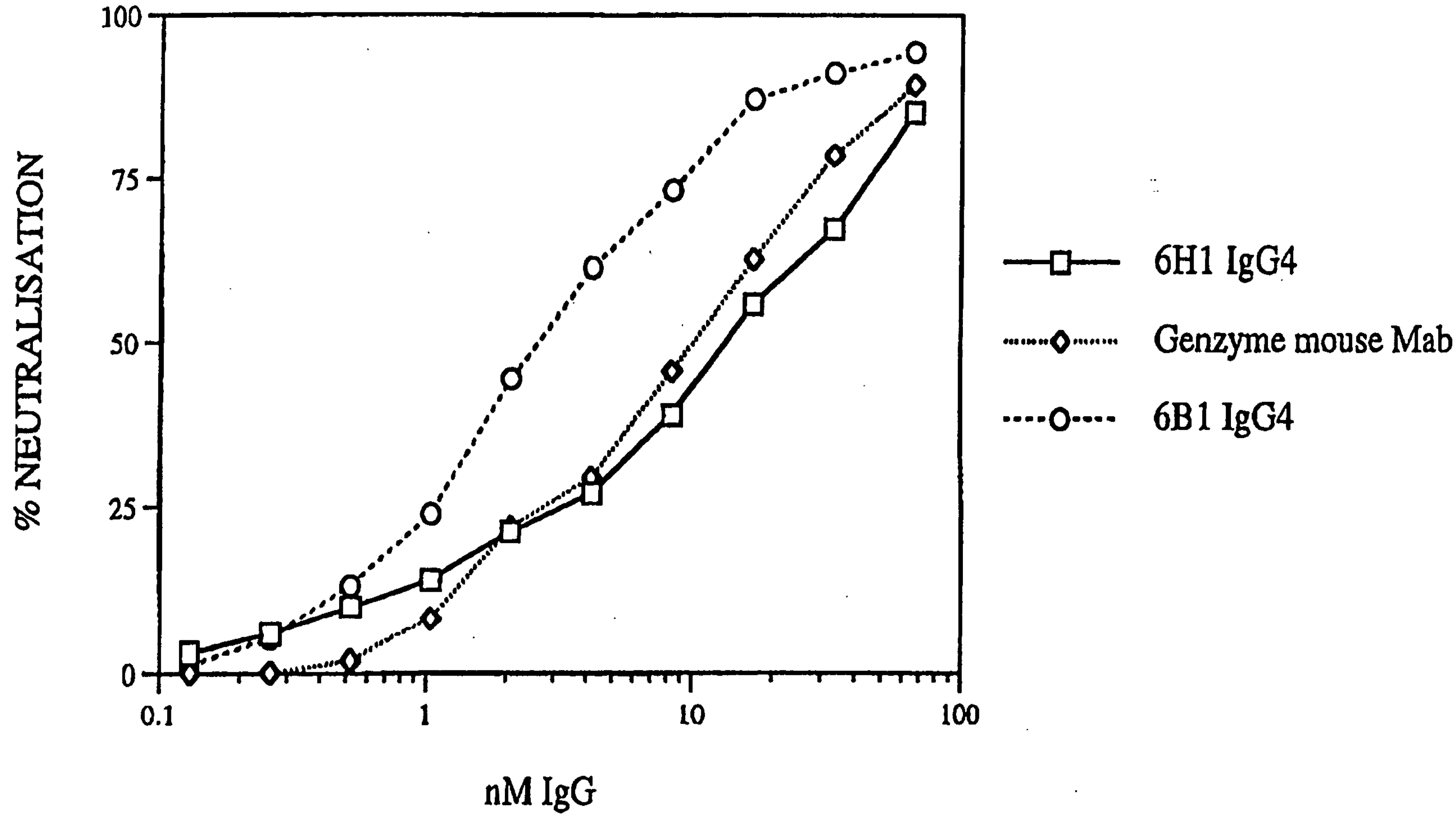


Figure 16

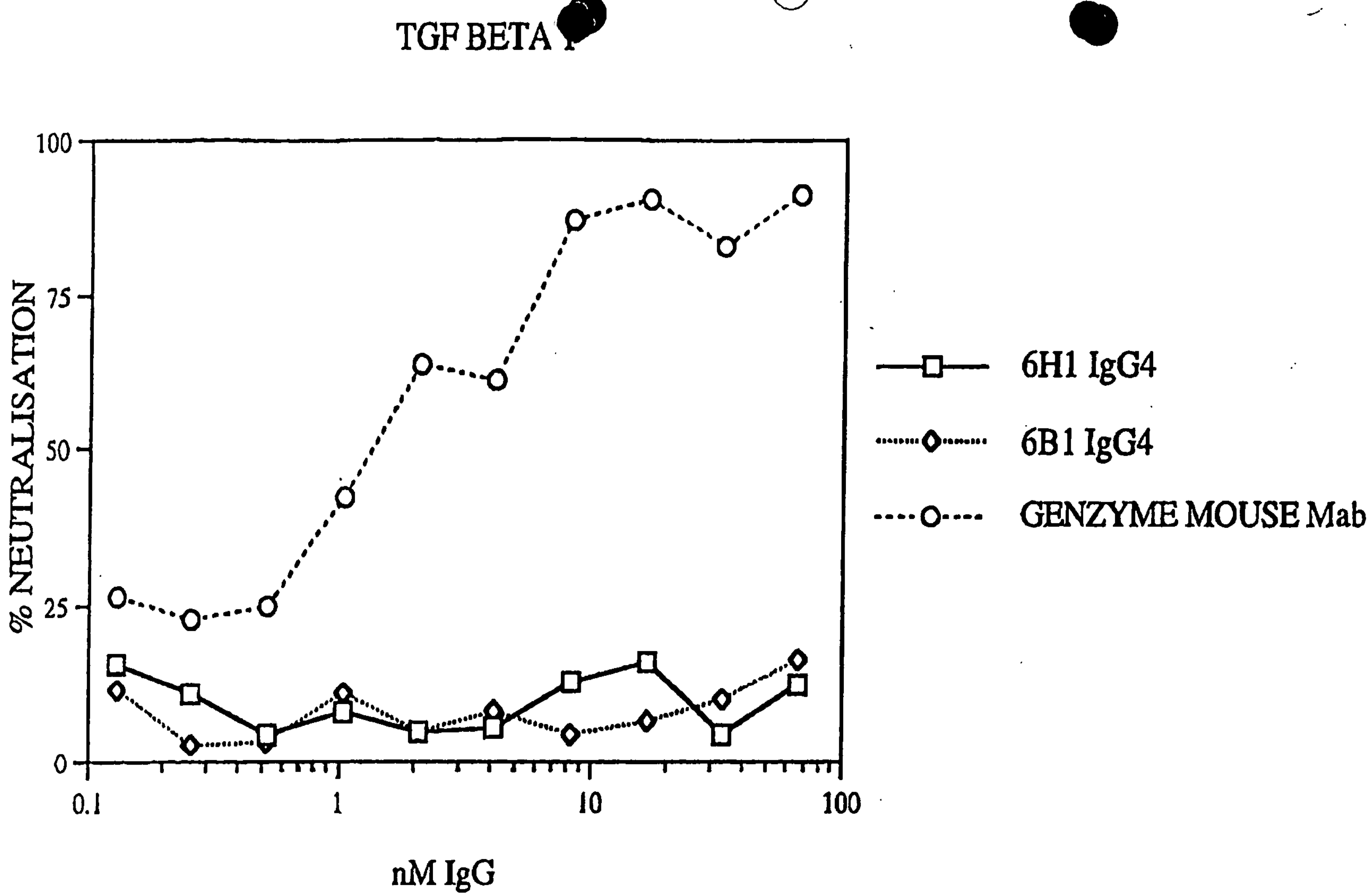

TGF BETA 1
% NEUTRALISATION
100
75
50
25
0
0.1
1
10
100
nM IgG
6H1 IgG4
6B1 IgG4
GENZYME MOUSE Mab

Figure 17

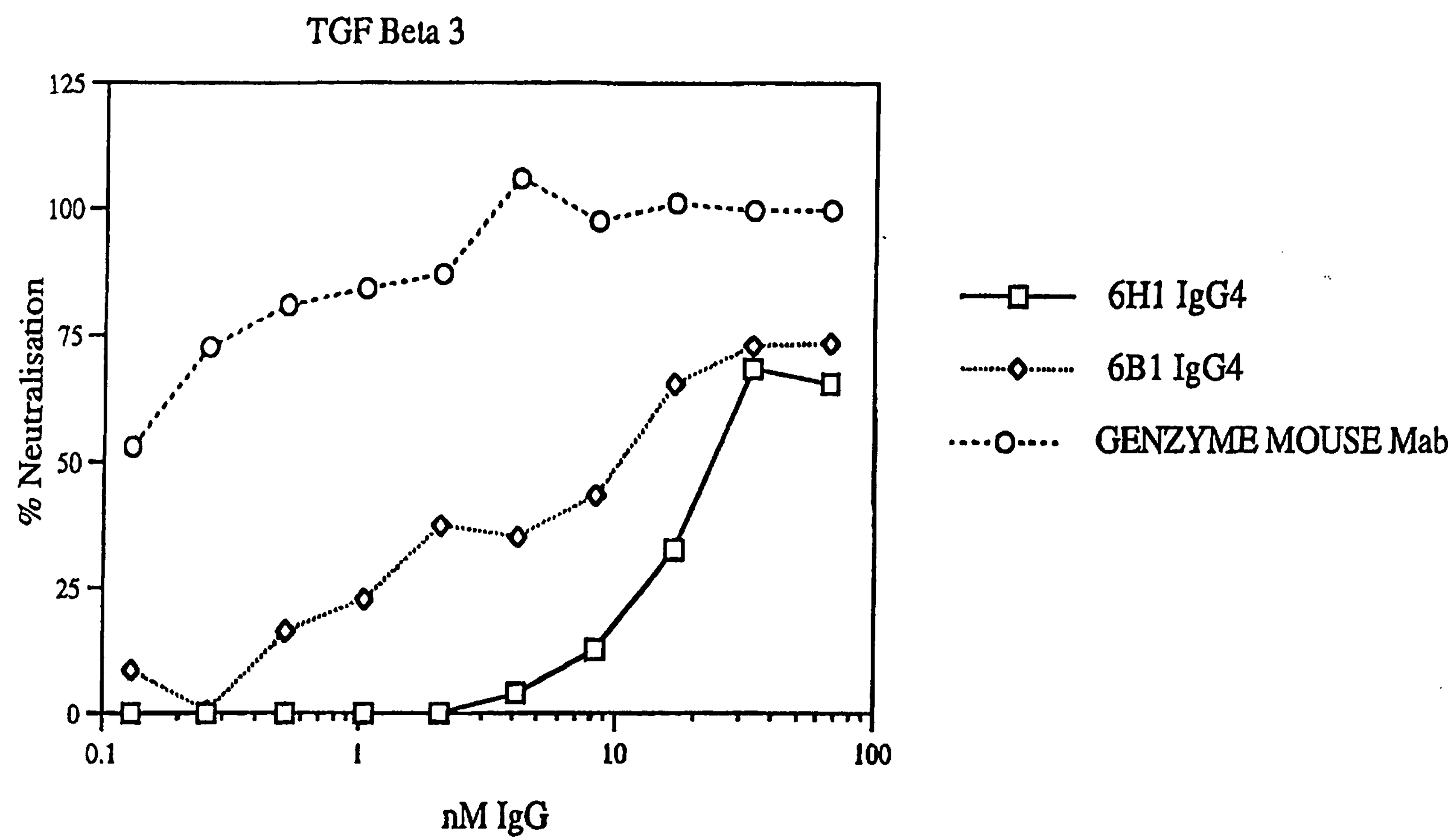

TGF Beta 3
125
100
75
50
25
0
% Neutralisation
0.1
1
10
100
nM IgG
6H1 IgG4
6B1 IgG4
GENZYME MOUSE Mab

Figure 18

Figure 19 Sequences of antibodies directed against TGFbeta2 showing CDR sequences in italics

2A-H11 VH (also known as 6H1 VH)

Sequence Range: 1 to 345

```
               10             20             30             40
        *       *      *       *      *      *      *       *      *
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

50             60             70             80             90
 *      *      *       *      *      *      *      *      *      *
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

  100            110            120            130            140
   *      *      *       *      *      *      *      *      *
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

    150            160            170            180            190
*      *       *      *      *      *      *      *      *      *
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

       200            210            220            230            240
  *      *      *      *      *      *      *      *      *      *
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>
```

```
                250        260        270         280
       *      *     *     *     *     *     *     *     *
CTG CAA ATG GAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   D   S   L   R   A   E   D   T   A   V   Y   Y   C>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

290         300         310         320         330
 *     *     *     *     *     *     *     *     *     *
GGA AGA ACG CTG GAG TCT AGT TTG TGG GGC CAA GGC ACC CTG GTC ACC
 G   R   T   L   E   S   S   L   W   G   Q   G   T   L   V   T>
___a___a___a____TRANSLATION OF 6H1 VH.SEQ [A]__a___a___a___a___>

  340
   *     *
GTC TCC TCA
 V   S   S>
___a___a___>
```

6B1 VL

Sequence Range: 1 to 330

```
             10         20         30          40
    *     *     *     *     *     *     *     *     *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>
```

```
  100         110         120          130         140
   *    *      *     *     *     *      *     *     *
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

    150         160         170         180          190
 *   *     *     *    *     *     *     *     *     *
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

       200         210          220         230         240
  *     *     *     *     *     *     *     *     *     *
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

          250         260         270          280
     *     *     *     *     *     *     *     *     *
GAT GAG GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT AGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   S   T   H>
___a___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___a___>

290         300          310         320         330
 *     *     *     *     *     *     *     *     *
CGA GGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 R   G   V   F   G   G   G   T   K   L   T   V   L   G>
___a___a____TRANSLATION OF 6B1 VL.SEQ [A]__a___a___a___>
```

6A5 VL

Sequence Range: 1 to 327

```
                 10          20          30          40
       *     *     *     *     *     *     *     *     *
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

 50          60          70          80          90
  *     *     *     *     *     *     *     *     *     *
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

    100         110         120         130         140
     *     *     *     *     *     *     *     *     *
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

       150         160         170         180         190
 *     *     *     *     *     *     *     *     *     *
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC GCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   A   G   S>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

          200         210         220         230         240
  *     *     *     *     *     *     *     *     *     *
AAC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAG
 N   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>
```

```
      250         260         270         280
       *     *     *     *     *     *     *     *     *
GAT GAG GCT GAC TAT TAC TGT AGC TCC CGG GAC AGC AGT GGT AAC CAT
 D   E   A   D   Y   Y   C   S   S   R   D   S   S   G   N   H>
___a___a___a____TRANSLATION OF 6A5 VL.SEQ [A]__a___a___a___a___>

290         300         310         320
 *     *     *     *     *     *     *     *
GTG GTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   V   F   G   G   G   T   K   L   T   V   L   G>
___a___a__TRANSLATION OF 6A5 VL.SEQ [A]____a___a___>
```

6H1 VL

Sequence Range: 1 to 348

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            10          20          30          40
       *     *     *     *     *     *     *     *     *
GAT GTT GTG ATG ACT CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA
 D   V   V   M   T   Q   S   P   S   S   L   S   A   S   V   G>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

50          60          70          80          90
 *     *     *     *     *     *     *     *     *     *
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGC ATT AGC AAT TAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

   100         110         120         130         140
    *     *     *     *     *     *     *     *     *
TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>
```

```
150         160         170         180         190
 *    *     *     *     *     *     *     *     *     *
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGT GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

        200         210         220         230         240
  *     *     *     *     *     *     *     *     *     *
AGT GGA TCT GGG ACA GAA TTC ACT CTC ACA ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   E   F   T   L   T   I   S   S   L   Q   P>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

            250         260         270         280
      *     *     *     *     *     *     *     *     *
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT CGA
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   R>
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>

290         300         310         320         330
 *     *    *     *     *     *     *     *     *     *
ACG TTC GGC CAA GGG ACC AAA GTG GAT ATC AAA CGT
 T   F   G   Q   G   T   K   V   D   I   K   R
___a___a___a____TRANSLATION OF 6H1 VL.SEQ [A]__a___a___a___a___>
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